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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/68, 33/573

A1

(11) International Publication Number: **WO 00/22437**

(43) International Publication Date: 20 April 2000 (20.04.00)

(21) International Application Number: PCT/US99/20698

(22) International Filing Date: 9 September 1999 (09.09.99)

(30) Priority Data:
09/172,521 14 October 1998 (14.10.98) US

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(81) Designated States: JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PROGNOSTIC METHOD FOR ANTI-RESORPTIVE TREATMENT

(57) Abstract

The present invention describes a new model based on the logistic combination of the percentage change of the level of a bone marker at a predetermined time period and the level of the bone marker at a baseline or at the predetermined period to predict shortly after initiating an anti-resorptive therapy those patients who will not significantly improve their bone BMD after two years of treatment or those patients who do not comply with therapy. In addition, the present invention also provides for the first time a defined cut-off value that can easily be used in clinical practice to identify individual non-responder or non-compliant patients after a short period of treatment, such as six months.

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PROGNOSTIC METHOD FOR ANTI-RESORPTIVE TREATMENT

Background of the Invention

Field of the Invention

The invention relates generally to anti-resorptive treatments of postmenopausal women, and specifically to methods of assessing the long-term efficacy of anti-resorptive treatments in individual postmenopausal women.

Description of the Prior Art

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Osteoporosis is a disease characterized by a low bone mass and architectural deterioration of bone tissue. Osteoporosis leads to increased susceptibility to fracture. Decreased bone mass is one of the main determinants of fracture. Two reasons may cause decreased bone mass: first, an imbalance between bone resorption and bone formation within a remodeling unit due to increased osteoclastic activity and/or decreased osteoblastic activity; second, an increase in the activation frequency, i.e., in the number of remodeling units initiated per unit of time and space. Increased bone turnover resulting from postmenopausal estrogen deficiency is the main determinant of bone loss and can be non-invasively assessed by measuring through serum and/or urine biochemical markers of bone turnover (1,2).

Anti-resorptive therapy, such as estrogen replacement therapy (HRT) and bisphosphonate treatment, have been shown to decrease bone turnover, preventing postmenopausal bone loss and significantly reducing fracture risk both in early and late postmenopausal women (3-6). After two years of anti-resorptive therapy, there is usually a small gain in bone mass in the order of 5% to 10% at the lumbar spine and less than 5% at the femoral neck and forearm. However, the precision error of bone mass measurement of the lumbar spine is about 1% to 2%, even by the most precise techniques, e g., dual or single energy X-ray absorptiometry. Therefore, it is usually necessary to wait up to two years after initiating therapy to determine in a single patient if a treatment is

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effective, i.e., increasing bone mass significantly. In most instances, repeating bone mass measurement at a shorter interval may not be helpful for the physician's decision making about compliance and treatment efficacy.

Conversely, anti-resorptive therapy induces a 30% to 60% decrease of markers of resorption and formation that fall within the premenopausal range within only three to six months (7). Thus, several studies have suggested that changes in bone markers after three to six months of treatment could be used to predict changes in bone mineral density (BMD) after one to two years in postmenopausal women treated either with estrogen (7-10) or bisphosphonate (11). In these studies the predictive values of markers was investigated in terms of a correlation between percentage change in markers after three to six months and percentage changes in bone mineral density at one or two years. Reported correlation coefficients are typically of 0.4-0.6, indicating that less than 40% of the inter-individual variance in long-term BMD changes can be predicted from early changes in bone markers. Obviously, this strategy cannot be used to accurately determine the absolute level of bone mass gain in individual patients. However, for the clinician, the primary concern is the fast detection of non-responders, i.e., patients who will fail to demonstrate a significant increase in BMD after one to two years of treatment, either resulting from poor compliance, non-absorption of the drug or no response for unknown reasons. By using marker percentage change from a baseline at six months in a receiving-operating characteristic (ROC) curve analysis model, Rosen et al. (10) recently showed that BMD responders to one year HRT can be predicted with a specificity of 90% but with a sensitivity of only 50%. No such analysis has been reported for bisphosphonate treatment.

Therefore, it is desirable to develop a method useful for predicting bone mass response and compliance of an individual after an anti-resorptive therapy, particularly a bisphosphonate treatment. It is also desirable to develop a method that optimizes the prediction in the early stage of treatment.

Summary of the Invention

It is an object of the present invention to provide a method useful for predicting bone mass response and compliance of an individual undergoing an anti-resorptive therapy. It is also an object of the present invention to develop a method that optimizes predictions in the early stage of the anti-resorptive therapy such that the prediction is specific and sensitive.

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These and other objects and advantages are achieved by the methods of the present invention. One aspect of the present invention provides a method for predicting bone mass response and compliance of an individual after an anti-resorptive therapy. In accordance with the present invention, the method basically includes the following steps:

1. measuring a baseline of a bone marker, *i.e.*, serum bone alkaline phosphatase (BAP), in the individual at the beginning of the anti-resorptive therapy;
2. measuring the level of the bone marker in the individual after a predetermined time period of the anti-resorptive therapy, *i.e.*, after six (6) months of therapy; and
3. generating a probability of response in bone mass after a second predetermined period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker either at the baseline or at the first predetermined time period.

According to one embodiment of the present invention, a logistic algorithm based on both the level of bone marker after a first predetermined time period and the change of bone marker level from the baseline is utilized to predict a probability of response in bone mass after a longer predetermined period of therapy, *i.e.*, two (2) years. According to another embodiment of the present invention, a logistic algorithm based on both the level of bone marker at the baseline and the change of bone marker level from the baseline after a predetermined period is utilized to predict a probability of response in bone mass after a longer predetermined period of therapy, *i.e.*, two (2) years.

In implementing the present invention method, a cutoff can be selected by a receiver-operating characteristic (ROC) curve analysis to provide corresponding sensitivity and specificity. The cutoff is a function of the change of the bone marker level at a predetermined time period and the level of bone marker at either the baseline or the predetermined time period. The present invention method utilizes a logistic regression model.

As provided by the present invention, the BMD data may be plotted on a two-dimensional diagram. One dimension of the two-dimensional diagram is bone marker level, and the other dimension the change of bone marker level from the baseline.

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Methods of the present invention provide a number of advantages. As explained in greater detail below, it has been found that methods of the present invention can quickly and accurately identify non-responders from responders to anti-resorptive treatment. For example, it can provide about 72% of sensitivity to predict two-year lumbar spine BMD response or to distinguish placebo from alendronate-treated patient for a given 90% specificity. Therefore, the methods of the present invention provide higher diagnostic specificity and sensitivity than the methods which consider the BAP level and BAP change parameters alone. In addition, by using the methods of the present invention, non-responders (or patients who do not adequately take alendronate) can easily be identified using a simple two-scale graph. In clinical practice, the identification of such patients after a few months of treatment would be useful to insure that alendronate is taken appropriately. Conversely, identification of a positive response to alendronate might improve long-term compliance.

The methods of the present invention are well suited for use during the anti-resorptive treatment for assessing the treatment response and the compliance of the treatment. They may also be applied to other markers of bone formation and bone resorption with different characteristics in terms of reproducibility and responses to alendronate. Furthermore, the methods of the present invention may also be applied to other anti-resorptive therapies such as estrogen replacement therapy. Finally, the methods of the present invention may be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss.

The invention is defined in its fullest scope in the appended claims and is described below in its preferred embodiments.

Description of the Figures

The above-mentioned and other features of this invention and the manner of obtaining them will become more apparent, and will be best understood, by reference to the following description, taken in conjunction with the accompanying drawings. These drawings depict only a typical embodiment of the invention and do not therefore limit its scope. They serve to add specificity and detail, in which:

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FIGURE 1 is a diagram which shows the response of bone alkaline phosphatase to treatment with alendronate (10 mg/day) or placebo in 307 elderly osteoporotic women.

FIGURE 2 is a diagram which shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level after six months of treatment) and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Responders are identified as patients with a percent increase in BMD from baseline after twenty-four months of treatment ≥ 3%. No BMD change was defined as a percent BMD change between –3% and +3%. BMD non-responders were women with a bone loss greater than 3%. The predicted probabilities were computed for differentiating BMD responders from both no BMD change and BMD non-responders considered as a single group.

FIGURES 3A and 3B are diagrams which show the areas under the ROC curve for the prediction of BMD (Fig. 3A) and alendronate-treated patients (vs. placebo, Fig. 3 B) in 307 elderly osteoporotic women. Three predictive models were compared: BAP percent change from baseline, BAP level, and their combination by logistic regression. ROC curves were established for the three discriminants and the areas under the curves computed as a function of the treatment monitoring time. P-values refer to the significance level of the difference between those areas as indicated.

FIGURE 4 is a diagram which shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (after six months of treatment) or their combination by logistic regression in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group.

FIGURE 5 is a diagram which shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level at the baseline and the predicted probability of lumbar BMD positive

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response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women.

FIGURE 6 is a diagram which shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (at the baseline) or their combination by logistic regession in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group.

Detailed Description of the Invention

The present invention is based on the discovery of a new model, based on the logistic combination of the level of bone marker and its percentage change in a patient at six months, to predict soon after initiating alendronate therapy those patients who will not significantly improve their BMD after two years of treatment. The present invention is also based on the discovery of a defined cutoff value that can easily be used in clinical practice to identify individual non-responder patients after only six months of treatment.

Accordingly, one aspect of the present invention provides a method for predicting a treatment response and compliance of an individual after an anti-resorptive therapy. The method comprises the steps of:

a. measuring a baseline of a bone marker in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of the bone marker in the individual after a first predetermined time period of anti-resorptive therapy; and

c. generating a probability of response in bone mass after a second predetermined time period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker at either the baseline or at the first predetermined time period for the purpose of determining the treatment response and compliance.

For the purpose of the present invention, an anti-resorptive therapy is a therapy that decreases bone turnover, prevents bone loss and reduces fracture

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risk. Examples of an anti-resorptive therapy include, but are not limited to, bisphosphonate therapy or estrogen replacement therapy (HRT). In accordance with one embodiment of the present invention, the anti-resorptive therapy is alendronate treatment therapy.

A bone marker may be any biochemical compound that can be used as a mark to reflect any changes in bone formation and bone resorption. Therefore, a bone marker can be any marker of bone formation and bone resorption. In accordance with one embodiment of the present invention, a bone alkaline phosphatase (BAP) is used as a bone marker. BAP is from serum; although, other bone markers from other sources derived from human body parts may also be used, e.g., urine samples.

A level of a bone marker in a patient may be measured by using conventional methods that are known to those skilled in the art. For example, to measure the level of BAP in serum, a two-site immunoradiometric assay using two monoclonal antibodies directed against the human bone isoenzyme may be used. Such a two-site immunoradiometric assay is known to one skilled in the art, and procedures can be obtained from the manufacturer Hybritech Incorporated (San Diego, California). Other known methods for measuring bone markers include high performance liquid chromatography (HPLC), lectin precipitation, heat inactivation and immunoadsorption.

For the purpose of the present invention, the first predetermined time period is a time period within which the decrease of a bone marker has reached a plateau. Such time may vary depending on how fast a bone marker responds to a treatment. For an anti-resorptive therapy, this period may be three to six months. Preferably, for alendronate treatment, a BAP level at the six months treatment is measured. It should be understood that earlier time points may also be valuable, and one skilled in the art can readily determine the time period for measuring a bone marker without undue experimentation in view of the present disclosure.

A response in bone mass may be a change in bone mineral density (BMD). In accordance with one embodiment of the present invention, a change in BMD is used as a response in bone mass for the anti-resorptive therapy. Typically, bone mass from lumbar spine is measured although bone mass from other organs such as, but not limited to, femoral neck and forearm may also be measured.

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A second predetermined time period of the anti-resorptive therapy is a time that a response in bone mass is sufficiently significant so that the responsiveness of the individual undergoing the anti-resorptive therapy can be determined. For example, a BMD change at two years of anti-resorptive therapy may be used to indicate whether a patient is a responder to the treatment. It will be understood that different time points may also be valuable. Therefore, a responder is defined as an individual, for example a woman, demonstrating a BMD increase after two years of treatment of 3% or more. A BMD change between -3% and +3% is considered as no significant change and an individual with a bone loss greater than 3% is considered as a non-responder.

A probability of a response in bone mass after a second predetermined time period of the anti-resorptive therapy may be generated by a logistic algorithm, preferably, a logistic regression algorithm, based on a change of the bone marker level from the baseline at a first predetermined period and the level of bone marker at either the baseline or the first predetermined time period. A logistic algorithm may be used to compute the statistical significance levels of each parameter estimated in the logistic equation. A logistic algorithm model may be evaluated based on the maximum likelihood estimation and Chi-square tests. A logistic regression model is appropriate only when the predicted probability ((p)-level) associated with a Chi-square and the slopes of each variable in the logistic equation are statistically significant (for example, $p<0.05$). Such a predicted probability from the logistic regression may be used to distinguish positivity (i.e., the BMD response of a patient in an alendronate group) from negativity.

In accordance with one embodiment of the present invention, a cutoff may be selected to provide a corresponding sensitivity and specificity of the prediction of the present invention. In one embodiment, a cutoff may be established by a receiver-operating characteristic (ROC) curve analysis. In this embodiment, the cutoff (t) is a function of two variables, i.e., BAP percentage change from baseline and BAP level at either the baseline or at six months, according to the following logistic equation:

$$t = \frac{1}{1 + e^{-Z}} \qquad [1]$$

where

$$Z = a + b \times (\text{change}) + c \times (\text{level}) \qquad [2]$$

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and a, b and c are logistic regression parameter estimates. Equation [1] can be transformed as:

$$\text{level} = -\frac{a - \log\frac{t}{1-t}}{c} - \frac{b}{c} \times (\text{change}) \quad [3]$$

Equation [3] indicates that for any particular t value, the cutoff corresponds to a straight line when patient's bone marker data is reported in two-dimension scatter-plots where axes represent the two variables, i.e., BAP percentage change from baseline (X) and absolute BAP level at either baseline or at six months (Y). This straight line separates positive from negative data points with a sensitivity and specificity that are set when the cutoff value t is selected by ROC curve analysis.

For example, according to one embodiment of the present invention, the individual patient's BMD data can be scatter-plotted on a two-dimensional diagram, where one dimension is serum BAP level at six months, and the other is percentage change of serum BAP level at six months. For a particular cutoff value, it corresponds to a straight line on the diagram, which separates the responsive from non-responsive points with a sensitivity and specificity set when the cutoff value is selected by ROC curve analysis. Likewise, in accordance with another embodiment of the present invention, the individual patient's BMD data may be scatter-plotted on a two-dimensional diagram where one dimension is serum BAP level at the baseline and the other is percentage change of serum BAP level at six months. Here, the "responsive" or BMD "response" is defined as an increase of the BMD by three percent (3%) or more over the long-term, *i.e.*, two (2) years of the anti-resorptive therapy.

Therefore, described generally, another aspect of the present invention provides a method of predicting a treatment response of an individual after a therapy. The present invention method comprises the steps of:

a. measuring the baseline of a first variable comprising a biochemical marker in the individual at the beginning of therapy;

b. monitoring the level of the first variable in the individual undergoing the therapy over a second variable; and

c. deriving a probability of treatment response from at least the first order derivative of the first variable over the second variable and

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the first variable either at the baseline or at a time determined by the second variable.

One type of therapy is anti-resorptive therapy. An example of the biochemical marker is serum bone alkaline phosphatase (BAP). The second variable may be time duration or other varying factors such as dosage of medicine, *etc.* When the second variable is time, the first order derivative of the serum BAP level is simply the change of the serum BAP level over a period of time. In accordance with one embodiment, the first variable may be the BAP level at the baseline. In accordance with another embodiment of the present invention, the first variable may be the BAP level at the time the change is determined.

The present invention provides a new model using the logistic combination of both the actual value and the percentage change of a bone marker after a short-term treatment period to identify patients who will subsequently demonstrate a positive bone mass response. This model provides a higher diagnostic specificity and sensitivity than the two individual parameters used alone. Using this model, non-responders (or patients who do not comply with the treatment) can easily be identified using a simple two-scale graph.

The present invention also employs a cutoff based on a logistic regression model, including both percentage change of a bone marker from the baseline level and the bone marker level at a predetermined time. This combination allows a substantial increase in the sensitivity of predicting a positive bone mass response with a similar specificity when the two parameters are used alone.

The methods of the present invention are well suited for use during the anti-resorptive treatment for assessing the treatment response and the compliance of the treatment. They may be applied to different markers of bone formation and bone resorption with different characteristics in terms of reproducibility and responses to treatment. Furthermore, the methods of the present invention may also be applied to a variety of anti-resorptive therapies, such as estrogen or alendronate treatment. Finally, the methods of the present invention may be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss (1, 15, 16).

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The following examples illustrate one detailed implementation of the present invention method.

EXAMPLES

Subjects

Three hundred and seven (307) women, aged 45-78 years (mean age: 64.0±7 years), were studied. They were all at least five years past a natural menopause (mean 17.6±8.0 years), and had lumbar spine BMD measured by dual energy X-ray absorptiometry (DXA) more than 2.5 standard deviation (SD) below the normal mean for premenopausal women. These late-postmenopausal osteoporotic women were enrolled in a two-year, double-blind, placebo-controlled trial, where the bisphosphonate alendronate (4-amino-l-hydroxybutylidene-1,1-bisphosphonate, Merck, USA) was administered orally once daily in the morning. Analysis was restricted to patients on placebo and to those treated with 10mg/day alendronate, i.e., the dose which is approved for the treatment of postmenopausal osteoporosis. All subjects also received 500 mg/day of elemental calcium (as carbonate).

Blood samples for biochemical marker measurements were obtained at the baseline, three, six, twelve and twenty-four months after initiation of therapy. They were stored at -20 C until assayed. All samples collected from a single subject throughout the study period were measured in a single assay run. Lumbar spine BMD was determined by DXA at the baseline and at twenty-four months of treatment, using a Hologic QDR-1000 densitometer (Hologic, Waltham, USA). The change of BMD with time was expressed in percentage change from the baseline. The study was approved by the ethics committees of the participating medical centers.

Measurement of Serum Bone Alkaline Phosphatase (BAP)

Serum BAP was measured with a human specific two-site immunoradiometric assay using two monoclonal antibodies directed against the human bone isoenzyme. BAP purified from human SAOS-2 osteosarcoma cells was used as a standard (Ostase®, Hybritech Incorporated, San Diego, California). In this assay, monoclonal antibodies cross-react by only 16% with the circulating liver isoenzyme. The sensitivity of the assay is 0.2 ng/ml, and the

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intra and inter assay coefficient of variation (CV) are less than 7% and 9%, respectively (12). The premenopausal range was established in one hundred thirty-four (134) healthy premenopausal (mean age: 41±5 years) women, belonging to a prospective population-based cohort (OFELY study: 1039 healthy volunteers, 31-89 years). The premenopausal range was 8.7±2.7 mg/L (2).

Statistical Analysis

Long-term variability of BAP measurement within a patient was assessed by the within-subject coefficient of variation on the five samples collected from the placebo group (N=175) over a twenty-four months period. The changes in biochemical markers of bone turnover with time under the treatment with alendronate and placebo were evaluated by analysis of variance. The correlation coefficients between the percentage change in lumbar spine BMD at month 24, absolute level of BAP, and the percentage change of BAP at month 6 were assessed by simple and multiple regression analysis.

Logistic Regression Model to Predict Long-term BMD Response and Compliance by BAP Level at Either Baseline or Six Months and Percentage BAP Change at 6 Months. Logistic regression was used to compute the probability of each patient to be in the alendronate-treated group or to predict their spine BMD response as a function of BAP percentage change from baseline and BAP level at three, six, twelve or twenty-four months of the treatment. Response to therapy was defined according to the percentage change from baseline in spine BMD after twenty-four months. As this variable is continuous, it has been re-coded as a binary variable (response vs. non-response) for logistic regression. Given the precision error CV of bone mass measurement by DXA, i.e., around 1%, a change in BMD would be significant at the individual level if it exceeds 1.96×(square root of 2)×CV, i.e., 2.8%. Thus, responders were defined as women demonstrating a percentage BMD change of 3% or more after two years. Non-responders to treatment were considered as patients with a two-year BMD change lower than 3%. Maximum likelihood estimation and Chi-square tests were used to estimate the goodness of fit of the overall model, and the statistical significance levels of each parameter estimated in the logistic equation were computed. The logistic regression model was considered appropriate only when the probability (p)-levels associated with Chi-square and the slopes of each variable in the logistic equation were statistically significant ($p<0.05$). Predicted probabilities (p) from the logistic regression were used to distinguish positivity (BMD response or patient in the alendronate group) from negativity (non-BMD

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response or patient in the placebo group). Cutoffs which provide appropriate sensitivity and specificity were established by ROC curve analysis.

In this model the cutoff t is a function of two variables, i.e., BAP percentage change from baseline and BAP level, according to the following classical logistic equation:

$$t = \frac{1}{1 + e^{-Z}} \qquad [1]$$

where

$$Z = a + b \times (\text{change}) + c \times (\text{level}) \qquad [2]$$

and a, b and c are logistic regression parameter estimates. Equation [1] can be transformed as:

$$\text{level} = -\frac{a - \log\frac{t}{1-t}}{c} - \frac{b}{c} \times (\text{change}) \qquad [3]$$

Equation [3] indicates that for any particular t value, the cutoff corresponds to a straight line when patients' bone marker date is reported in two-dimension scatter-plots where axes represent the two variables, BAP percentage change from baseline (X) and absolute BAP level at either the baseline or at six months (Y). This straight line separates positive from negative data points with a sensitivity and specificity that are set when the cutoff value t is selected by ROC curve analysis.

Comparison of the Different Models of Prediction. The model based on logistic regression analysis which combines both BAP level at either the baseline or six months and BAP percentage change at six months was compared with models using either one of these two individual discriminants. Overall discriminant performances were compared in terms of area under the ROC curve. Comparisons were also performed for a given threshold of specificity by paired Chi-square tests.

RESULTS

Effect of Alendronate Treatment on BAP Levels and Relationships with BMD Changes.

At baseline, BAP levels were increased by a mean 95% compared to premenopausal values and 68.4% of patients had levels above the upper limit of

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premenopausal range (mean+2SD: 14.1mg/L) (data not shown). Upon alendronate treatment, BAP showed a progressive decrease reaching a nadir after six months of treatment (-44%), and the levels did not further change for the duration of the study (Fig. 1). Figure 1 shows a response of bone alkaline phosphatase to treatment with alendronate (10 mg/day) or placebo in 307 elderly osteoporotic women. Data are the mean±1 SEM. After six months of alendronate treatment, 95% of values were within the premenopausal range.

The percentage change from baseline in spine BMD at twenty-four months correlated significantly with both BAP level at six months and the percentage change BAP at six months ($r = -0.61$ and -0.60, $p < 0.001$, respectively).

Combination of Level and Percentage Change of BAP at 6 Months to Monitor Alendronate Treatment

Based on the percentage change in spine BMD after two years of treatment (alendronate and placebo), 45% of women were classified as BMD responders (increase in BMD ≥3%), 39.7% as no BMD change (BMD change -3% to +3%), and 15.35 as non-responders (loss of BMD >3%). Figure 2 shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level after six months of treatment, and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Responders are identified as patients with a percent change in lumbar BMD from baseline after twenty-four months of treatment at or greater than 3%. The two latter groups, (i.e., no BMD change and non-responders) have a similar distribution of BAP levels at six months and BAP percentage change at six months, and could not be discriminated by these two parameters. Thus, these two groups were combined in the subsequent analyses, and the value of BAP levels, BAP percentage change at six months and the logistic combination of these two parameters were investigated to discriminate BMD responders (increase in BMD ≥3%).

Figure 5 shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP levels at the baseline, and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Likewise, responders are identified as patients with a percent change in lumbar BMD from baseline after twenty-four months of

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treatment at or greater than 3%. As shown in Figure 5, the two latter groups, (i.e., no BMD change and non-responders) have a similar distribution of BAP levels at the baseline and BAP percentage change at six months, and could not be discriminated by these two parameters. Thus, these two groups were combined in the subsequent analyses, and the value of BAP levels at the baseline, BAP percentage change at six months and the logistic combination of these two parameters were investigated to discriminate BMD responders (increase in BMD ≥ 3%).

Levels of BAP at either the baseline or at six months and percentage changes of BAP at six months were significant and independent predictors of BMD response at two years (i.e., with a BMD gain at two years of 3%, or more) in logistic regression analysis ($p < 0.02$ for both parameters). These two parameters were thus combined to compute the predicted probability of individual patients to respond to alendronate treatment (see statistical analysis Figure 2 and Figure 5). As expected, responders and non-responders have distinct distributions in relation to their predicted probabilities, the former group being characterized by higher probabilities of spine BMD gain at two years.

The area under the ROC curve was used to compare the discriminative power of this combination model with those using only BAP level at six months or percentage BAP change at six months. Figure 3 shows the area under the ROC curve for the prediction of BMD (Panel A) and alendronate-treated patients (vs. placebo, panel B) in 307 elderly osteoporotic women. Three predictive models were compared: BAP percent change from baseline, BAP level, and their combination by logistic regression. ROC curves were established for the three discriminants and the areas under the curves computed as a function of the treatment monitoring time. P-values refer to the significance level of the difference between those areas as indicated. As shown in Figure 3, the discrimination between BMD responders/non-responders (Fig. 3A) and alendronate-treated patients/placebo (Fig. 3B) increases with time after initiating therapy, a plateau being reached at six months for the prediction of BMD response. BAP percentage change from the baseline and the BAP level appear to be equivalent when used separately, except at three months when BAP level shows the lowest discrimination power. Irrespective of monitoring time, the discrimination power provided by logistic combination of BAP percentage change from baseline and BAP level is superior to that obtained with either of the two monitoring parameters taken separately.

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A cutoff can be selected by logistic regression and ROC curve analysis (Figs. 4 and 6) to provide after six months of treatment a specificity of 90%. Figure 4 shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (after six months of treatment) or their combination by logistic regression in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group. This cutoff implies that no more than 10% of women classified with markers as having a subsequent positive BMD response (i.e., an increase at two years > 3%) would be false positive. This cutoff results in a 72% sensitivity in the detection of patients who will demonstrate a favorable spine BMD increase after two years of treatment.

Figure 6 shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (form baseline after six months of treatment) or BAP level (at the baseline) or their combination by logistic regression in 307 elderly osteoporotic women. Likewise, discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group. This cutoff implies that no more than 10% of women classified with markers as having a subsequent positive BMD response (*i.e.*, an increase at two years > 3%) would be false positive. This cutoff results in a 73% sensitivity in the detection of patients who will demonstrate a favorable spine BMD increase after two years of treatment.

By comparison, BAP percentage change from baseline, BAP level at six months or BAP level at the baseline taken separately would provide significantly lower sensitivities for the same 90% specificity (Table 1 and Figure 6). Table 1 shows the sensitivity of different models to predict two-year lumbar spine BMD response or to distinguish placebo from alendronate-treated patients for a given

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90% specificity in each model. When patients with no significant BMD change (-3% < %BMD change < +3%) were excluded from the analysis, for the same 90% specificity, the sensitivity to detect responders increased slightly for each model and was also highest for the logistic regression combination (67%, 70%, and 75% for BAP level at six months, percentage change of BAP at six months and the logistic combination of BAP level and % BAP change, respectively) (data not shown). A cutoff can also be set to differentiate women on placebo from alendronate-treated patients by logistic regression and again the sensitivity obtained from the combination of BAP level and BAP percentage change at six months was higher than that obtained by each parameter alone (Table 1).

TABLE 1

	BMD response		Alendronate/placebo	
Predictive model	Cutoff	Sensitivity (%)	Cutoff	Sensitivity (%)
BAP level at 6 months	9.5µg/L	59V	.94 µg/L	66
% BAP change at 6 months	-38.2%	61	-38.5	71
BAP level at 6 months + % BAP change at 6 months	BAP level=1.7-0.28* % BAP change	72*	BAP level = 3.5-0.24* % BAP change	82*

Combination of BAP Percentage Change and BAP Level at 6 Months Compared to BAP Least Significant Change Cutoff for Treatment Monitoring

The long-term within patient variability of BAP levels over a twenty-four-month period in the placebo group (n=175) was 15.7%, resulting in a least significant change cutoff of 43.5%. When patients with BAP percentage change from baseline lower than –43.5% at six months of treatment are classified as BMD responders, BMD response can be predicted with a 93% specificity which is comparable to the 90% of the logistic model response at two years but with

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only 54% of sensitivity, which is significantly lower than the 72% obtained from the logistic combination ($p < 0.002$). Similarly, the least significant change cutoff is less powerful to differentiate placebo from alendronate- treated patients than the combination model with a 22% lower sensitivity (60% vs. 82%, $p<0.001$) for a similar specificity (95% vs. 90%, NS).

It might be argued that BAP least significant change calculated from the placebo group overestimates BAP variability in normal individuals. A value of 25% has been proposed as a cutoff of significant change of BAP (13). In this study, a cut-point of -25% BAP percentage change from baseline at six months would result in sensitivities for treated individuals or for positive BMD response prediction statistically similar to those observed with the logistic (85% vs. 82% and 78% vs. 72%, respectively). In both cases, however, resulting specificity would be much lower than that obtained from the logistic combination: 78% and 75% versus 90%, respectively ($p<0.001$).

DISCUSSION

The present invention provides a new model using the logistic combination of both an absolute level and the percentage change of a bone marker after a short-term treatment period to rapidly identify patients who will subsequently demonstrate positive BMD responses. This model gives higher diagnostic specificity and sensitivity than the two individual parameters considered alone. In addition, using this model, non-responders (or patients who do not adequately take alendronate) can easily be identified using a simple two-scale graph.

The present invention is based on the discovery that the combination of both the absolute level of BAP and the percentage change of BAP at the six-month date of an anti-resorptive therapy can be used to predict the probability of a positive BMD response after two years of the therapy, which responses is determinative of the effectiveness of the treatment. It is a discovery of the present invention that as the decrease in BAP reaches a plateau after six months of treatment, this six-month time point is the most adequate to test the usefulness of bone markers to predict bone mass response, although earlier time points may also be valuable (9-11). It is an observation of the present invention that there is a significant correlation between the percentage change of bone marker levels at six months and the percentage change of spine BMD at two years with a correlation coefficient slightly lower than that previously

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observed in a smaller study (n=75) with the same drug (-0.62 vs. -0.77). In addition, a strong negative correlation between absolute levels of bone markers at six months and percentage spine BMD changes at six months is also surprisingly observed, which suggests that this parameter could also be used to monitor the efficacy of alendronate treatment. Based on these discoveries and observations, the present invention combines these two parameters, i.e., percentage BAP change and BAP level, to improve the prediction of bone mass response.

In addition, it is another discovery of the present invention that the combination of both the absolute level of BAP at the baseline and the percentage change of BAP at the six-month date of an anti-resorptive therapy can be used to predict the probability of a positive BMD response after two years of the therapy. Based on this discovery, the present invention combines these two parameters, *i.e.*, BAP level at the baseline and percentage BAP change at six months to improve the prediction of bone mass response.

Using a cutoff based only on the percentage change from baseline of bone marker levels after thee to six months of treatment, i.e., when the decrease of bone turnover has reached a plateau, may not be sufficient to accurately identify patients who will respond favorably to treatment in terms of BMD gain after two years. Indeed, it seems reasonable to consider that patients who have relatively low bone marker levels before treatment may only demonstrate a slight decrease in bone marker and might not be identified as responders despite levels at six months within the normal range, demonstrating the efficacy of the treatment to normalize bone turnover. Therefore, a parameter which represents the level of bone turnover at either the baseline or at a level reached after treatment might be an additional and independent predictor of BMD response.

Based on these beliefs and observations, the present invention performed logistic regression, including both percentage change from baseline and BAP level at either the baseline or at six months to predict BMD responders. It is found that these two parameters are significant and independent predictors of BMD response and their combination provides a significantly higher predictive value than each parameter alone. For example, a high specificity, i.e., the proportion of non-responders who are identified by the predictive model at six months and who indeed did not demonstrate a significant gain in spine BMD at two years, is likely to be the most relevant option that could lead to therapeutic adjustment, including changing drug or dose. For a given 90% specificity, it is

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found that combining the percentage change and BAP level at six months, one could improve significantly the sensitivity by 11% to 13%, compared to using either of the two parameters alone. In addition, the predictive performance of the logistic model was compared with a cutoff based on the least significant change of BAP, which has been suggested as an adequate means tool to detect responders. Based on the five measurements performed on the 175 women of the placebo group, we estimated the least significant change as 43.5%, which is similar to the mean decrease in BAP after six months of alendronate treatment. Such a high least significant change calculated from the placebo group may overestimate BAP variability in normal individuals, because of calcium supplementation in placebos which is likely to decrease bone turnover. Therefore, a -25% cutoff was also used, which has been previously suggested as a more realistic least significant change for that marker (13). It was found that whatever the cutoff of least significant changes considered, the logistic combination model gave significantly higher predictive performance for BMD response or for differentiating placebo from alendronate-treated patients.

This model may be generalizable to other populations. Although this model was constructed from predicted probabilities which are as any predictive value dependent on the prevalence of positive cases in the study population used for their computation by logistic regression, the deduced cutoff lines used to differentiate between positive (BMD responder) and negative (non-responder) cases with defined sensitivity and specificity characteristics are independent of any prevalence. Thus, these cutoff lines determined in this large population are likely to be useful to provide corresponding sensitivity and specificity values in any population with comparable properties in terms of BAP response, even if expected prevalence of positive cases are different. It could also be argued that this predictive model may be more difficult to use than a single percentage BAP change parameter because it requires combining two parameters. However, as illustrated in Figures 4 and 6, non-responders can easily be distinguished from responders by reporting patient characteristics (% BAP change at six months and BAP level at six months or at the baseline) in a two-scale graph with the cutoff as a straight line.

This model was developed using samples from a placebo-controlled clinical trial and it is hypothesized that the biochemical changes observed in placebo-treated women would reflect changes observed in non-compliant patients. In clinical practice, the identification of such patients after a few

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months of treatment would be useful to insure that alendronate is taken appropriately. Conversely, identification of a positive response to alendronate might improve long-term compliance.

This model may be applicable to other markers of bone formation and bone resorption with different characteristics in terms of reproducibility and response and to other anti-resorptive therapies such as estrogen. This model could also be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss (14, 15).

The foregoing is meant to illustrate, but not to limit, the scope of the invention. Indeed, those of ordinary skill in the art can readily envision and produce further embodiments, based on the teachings herein, without undue experimentation.

The present invention may be embodied in other specific forms without departing from its essential characteristics. The described embodiment is to be considered in all respects only as illustrative and not as restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of the equivalence of the claims are to be embraced within their scope.

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What is claimed is:

1. A method for predicting a treatment response and compliance of an individual after an anti-resorptive therapy, comprising the steps of:

a. measuring a baseline of a bone marker in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of the bone marker in the individual after a first predetermined time period of anti-resorptive therapy; and

c. generating a probability of response in bone mass after a second predetemrined time period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker at either the baseline or at the first predetermined time for the purpose of determining the treatment response and compliance.

2. The method of claim 2, wherein the probability is generated by using a logistic algorithm.

3. The method of claim 2, wherein the logistic algorithm is a logistic regression algorithm.

4. The method of claim 1, wherein the bone marker is selected from a group consisting of markers of bone formation, and marks of bone resorption.

5. The method of claim 4, wherein the bone marker is serum bone alkaline phosphonate.

6. The method of claim 1, wherein the anti-resorptive therapy is bisphosphonate treatment therapy or estrogen replacement therapy.

7. The method of claim 6, wherein the anti-resorptive therapy is alendronate treatment.

8. The method of claim 1, wherein the first predetermined time period is about 3 to 6 months.

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9. The method of claim 8, wherein the first predetermined time period is about 6 month of anti-resorptive therapy.

10. The method of claim 1, wherein the second predetermined time period is about two years of the anti-resorptive theray.

11. The method of claim 1, further comprising a step of selecting a cutoff to provide corresponding sensitivity and specificity.

12. The method of claim 11, wherein said cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

13. The method of claim 11, wherein said cutoff is a function of the change of bone marker level and the level of bone marker at either the baseline or at the first predetermined time.

14. The method of claim 1, further comprising the steps of plotting the bone mass of the individual on a two-dimensional diagram.

15. The method of claim 14, wherein one dimension of the two-dimensional diagram is the bone marker level, and the other dimension of the two-dimensional diagram is the change of bone marker level.

16. The method of claim 1, wherein the bone mass is measured by bone mineral density (BMD).

17. The method of claim 16, wherein the response is defined as a BMD increase of three percent (3%) or more over the second predetermined time period of the anti-resorptive therapy.

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18. A method of predicting lumber spine bone mineral density (BMD) response and compliance of an individual after an anti-resorptive therapy, comprising the steps of:

a. measuring a baseline of serum bone alkaline phosphatase (BAP) in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of serum BAP in the individual after a first predetermined time period of the anti-resorptive therapy;

c. calculating a percentage change of serum BAP level from the baseline; and

d. determining a probability of a response in lumber spine BMD after a second predetermined time period of the anti-resorptive therapy by utilizing a logistic regression algorithm based on the percentage change of serum BAP level and the level of serum BAP at either the baseline or the first predetermined time.

19. The method of claim 18, further comprising a step of selecting a cutoff to provide a corresponding sensitivity and specificity.

20. The method of claim 19, wherein the cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

21. The method of claim 19, wherein the cutoff is a function of the percentage change of serum BAP level and the level of serum BAP at either the baseline or the first predetermined time.

22. The method of claim 18, wherein the algorithm employs logistic regression parameter estimates.

23. The method of claim 18, further comprising the step of plotting the lumber spine BMD of the individual on a two-dimensional diagram.

24. The method of claim 23, wherein one dimension of the two-dimensional diagram is the serum BAP level, and the other dimension of said two-dimensional diagram is the percentage change of the serum BAP level.

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25. The method of claim 18, wherein the first predetermined time period of the anti-resorptive therapy is six (6) months.

26. The method of claim 18, wherein the second predetermined time period of the anti-resorptive therapy is two (2) years.

27. The method as of claim 18, wherein the response is defined as an increase of the lumber spine BMD by three percent (3%) or more over the second predetermined time period of the anti-resorptive therapy.

28. A method of predicting a treatment response of an individual after a therapy, comprising the steps of:

a. measuring the baseline of a first variable comprising a biochemical marker in the individual at the beginning of the therapy;

b. monitoring the level of the first variable in the individual undergoing the therapy over a second variable; and

c. deriving a probability of treatment response from at least the first order derivative of the first variable over the second variable and the first variable either at the baseline or at a time determined by the second variable.

29. The method of claim 28, wherein the biochemical marker is serum bone alkaline phosphatase (BAP).

30. The method of claim 28, wherein the second variable is time.

31. The method of claim 30, wherein the at least the first order derivative of the first variable over the second variable is the change of the level of the first variable over a period of time.

32. The method of claim 31, wherein the period of time is six (6) months.

33. The method of claim 31, wherein the probability of a treatment response is predicted for a longer period of time.

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34. The method of claim 33, wherein the longer period of time is two (2) years.

35. The method of claim 28, wherein said treatment response is measured by a bone mineral density (BMD) response.

36. The method of claim 35, wherein said response is defined as a BMD increase of three percent (3%) or more over said long-term of said anti-resorptive therapy.

37. The method of claim 28, wherein said probability of treatment response is derived by utilizing a logistic regression algorithm.

38. The method of claim 37, further comprising a step of selecting a cutoff to provide corresponding sensitivity and specificity.

39. The method of claim 38, wherein said cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

40. The method of claim 38, wherein said cutoff is a function of at least the first order derivative of said first variable over said second variable and the first variable either at the baseline or at a time determined by the second variable.

41. The method of claim 28, further comprising the steps of plotting said treatment response of said individual on a two-dimensional diagram.

42. The method of claim 41, wherein one dimension of said two-dimensional diagram is said first variable, and the other dimension of said two-dimensional diagram is said at least the first order derivative of said first variable over said second variable.

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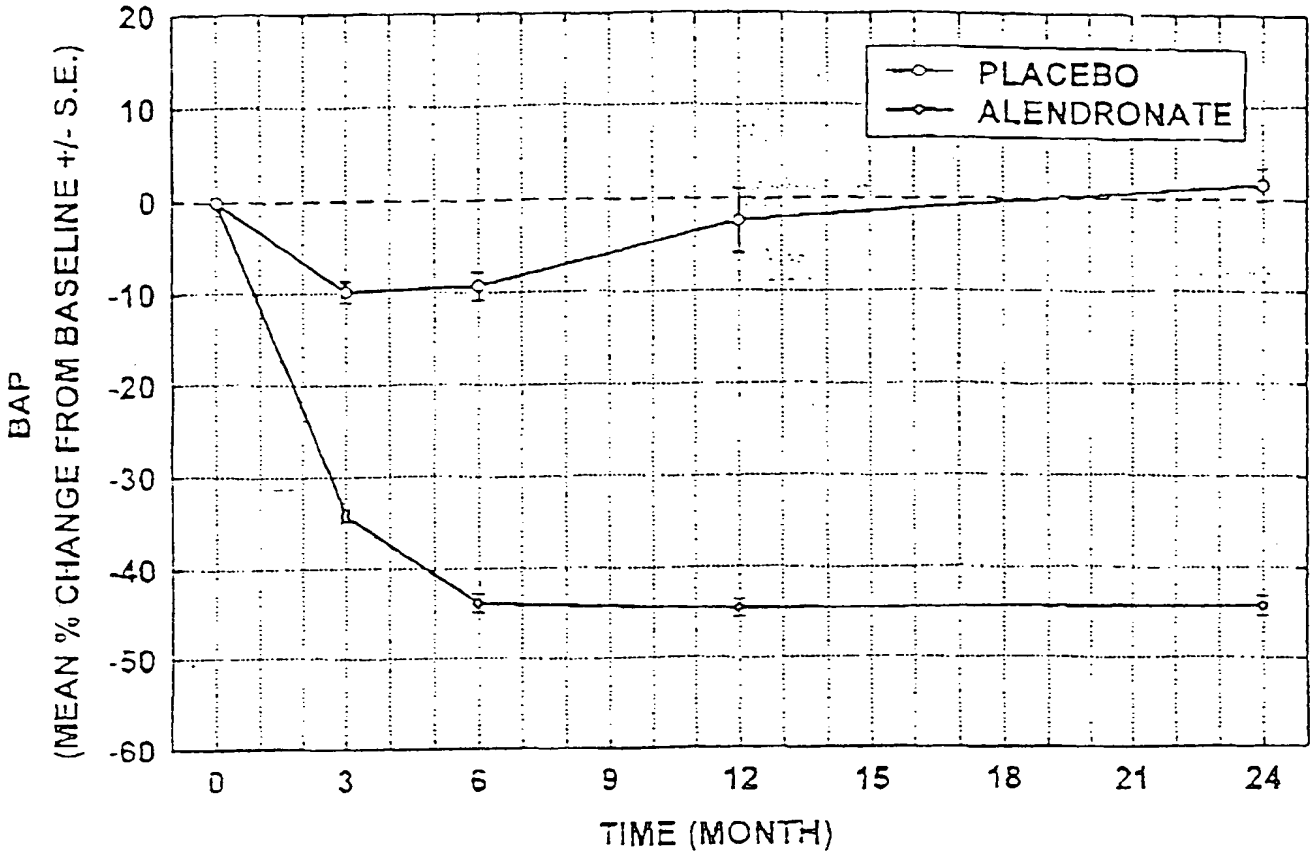

Fig. 1

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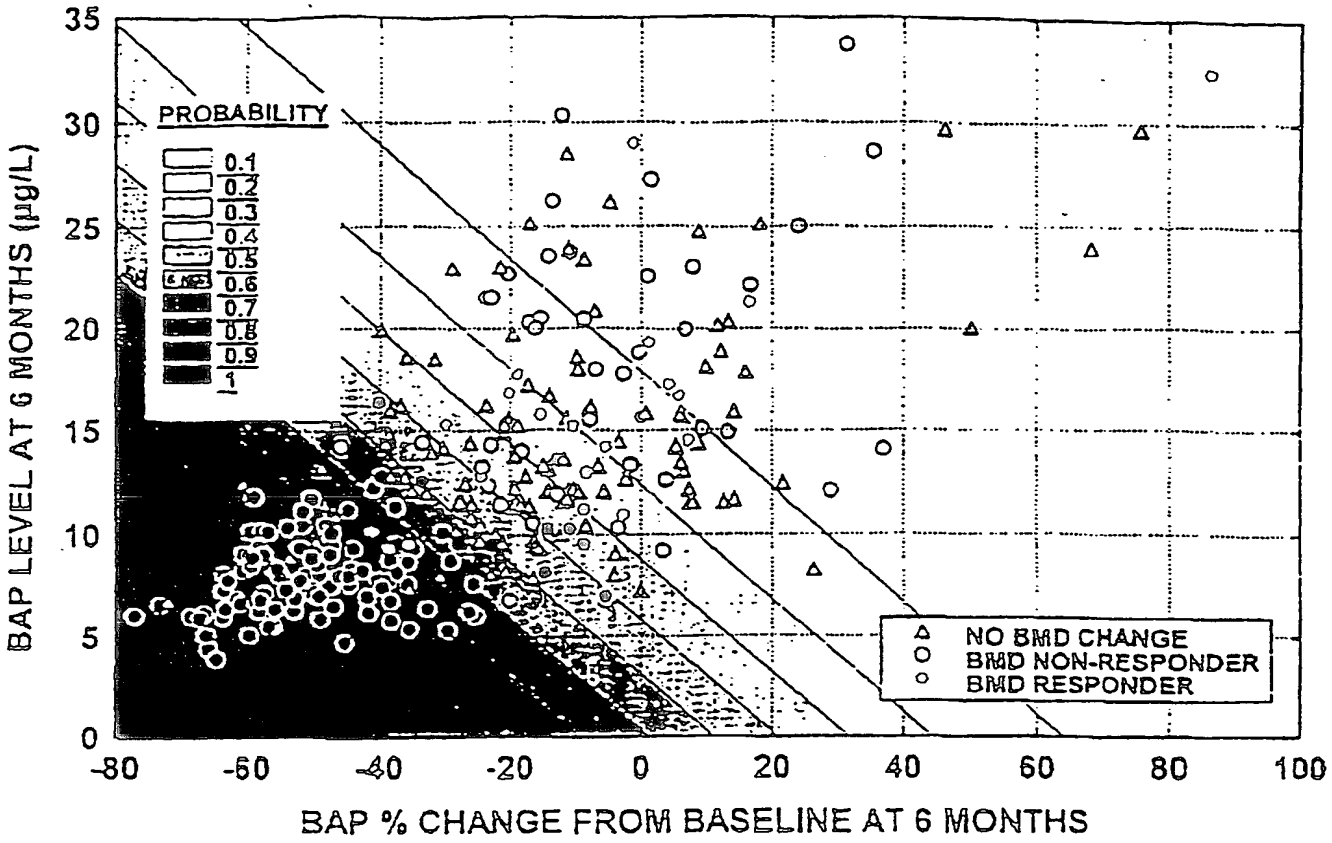

Fig. 2

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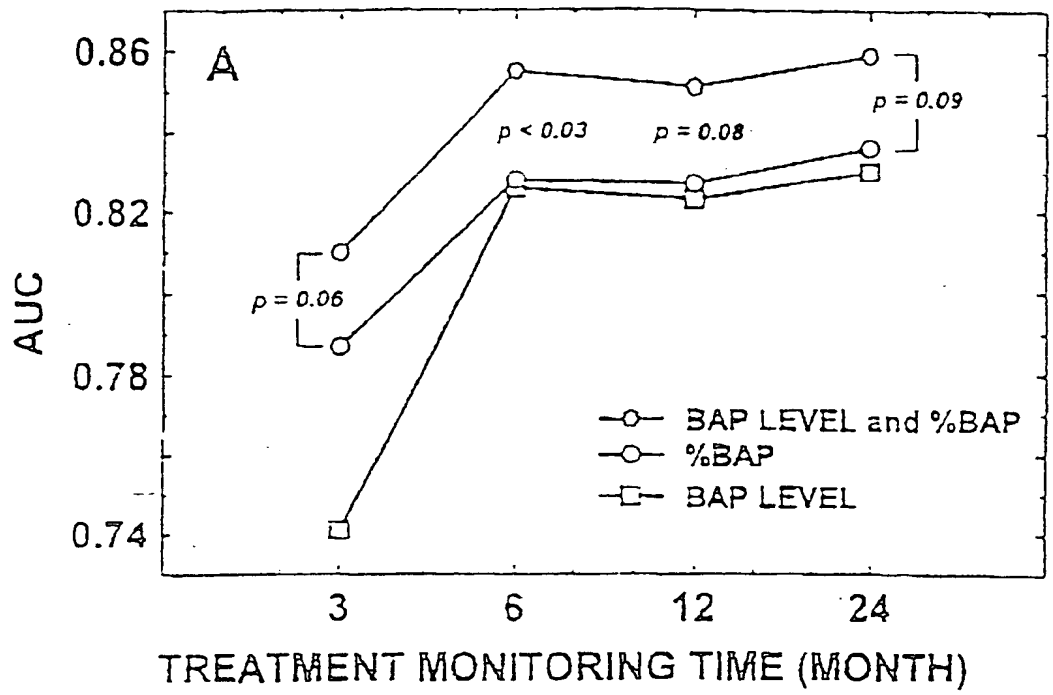

Fig. 3A

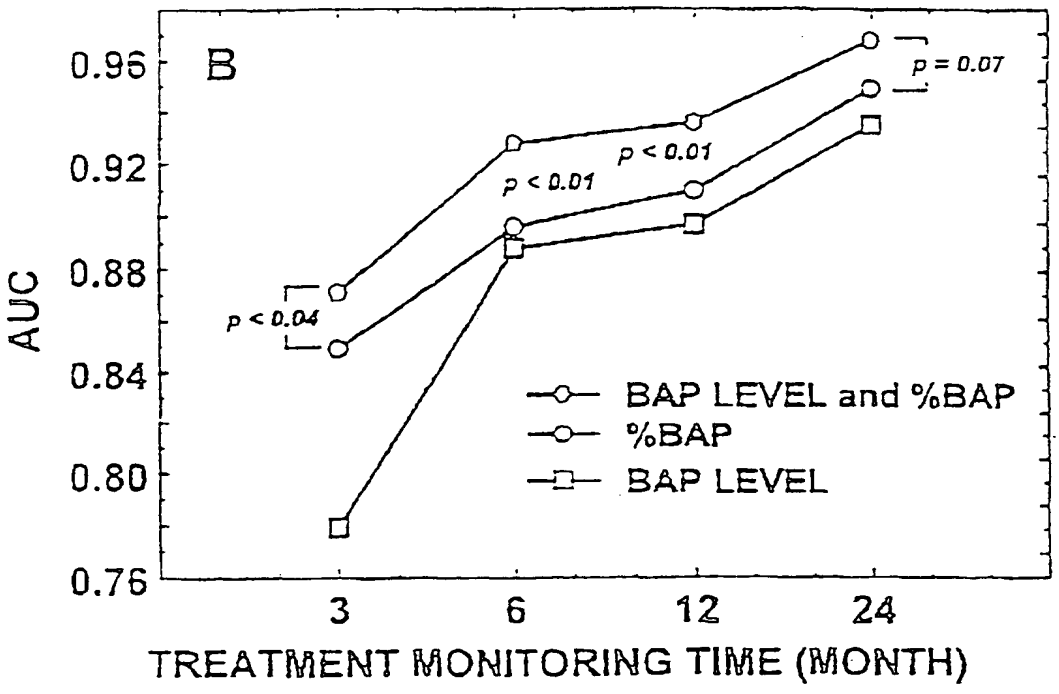

Fig. 3B

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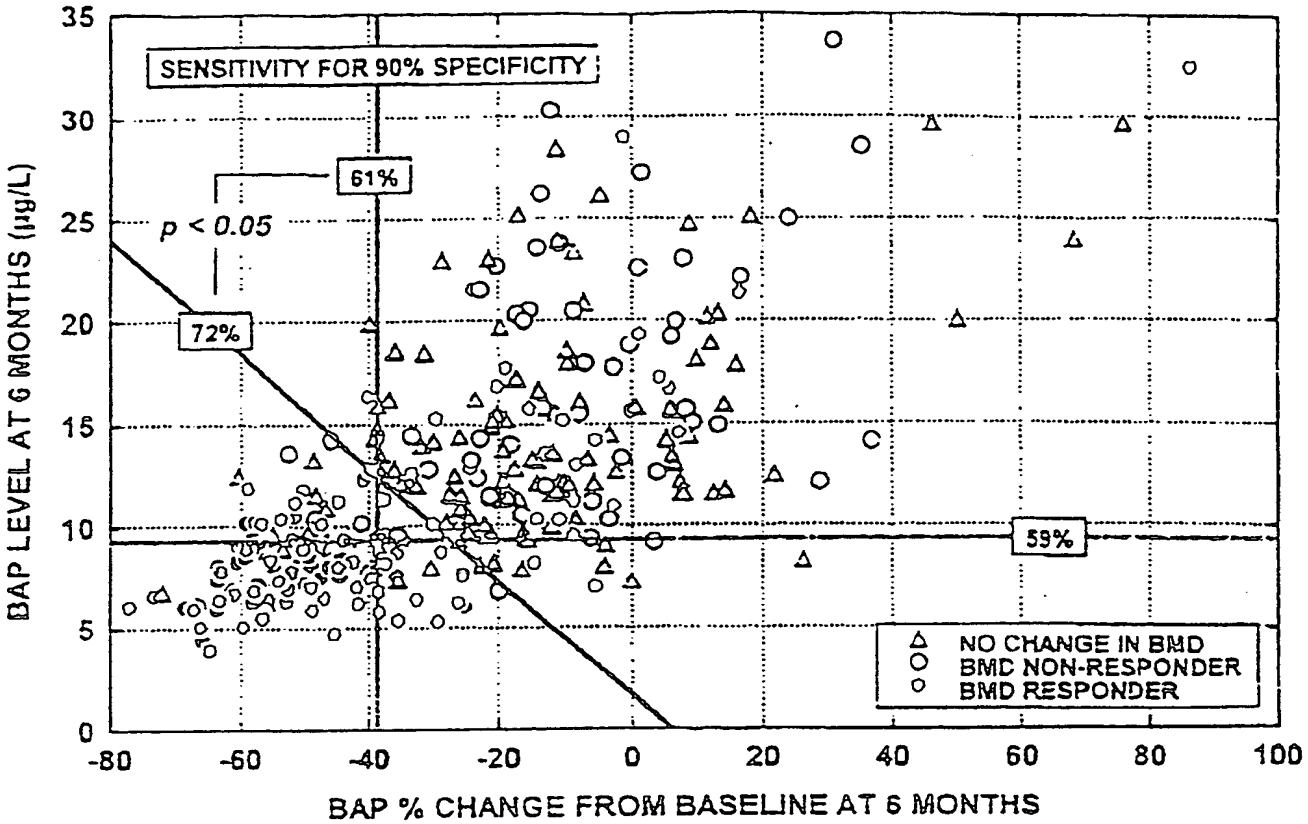
SENSITIVITY FOR 90% SPECIFICITY
61%
p < 0.05
72%
59%
NO CHANGE IN BMD
BMD NON-RESPONDER
BMD RESPONDER
BAP LEVEL AT 6 MONTHS (µg/L)
BAP % CHANGE FROM BASELINE AT 6 MONTHS
35
30
25
20
15
10
5
0
-80
-60
-40
-20
0
20
40
60
80
100

Fig. 4

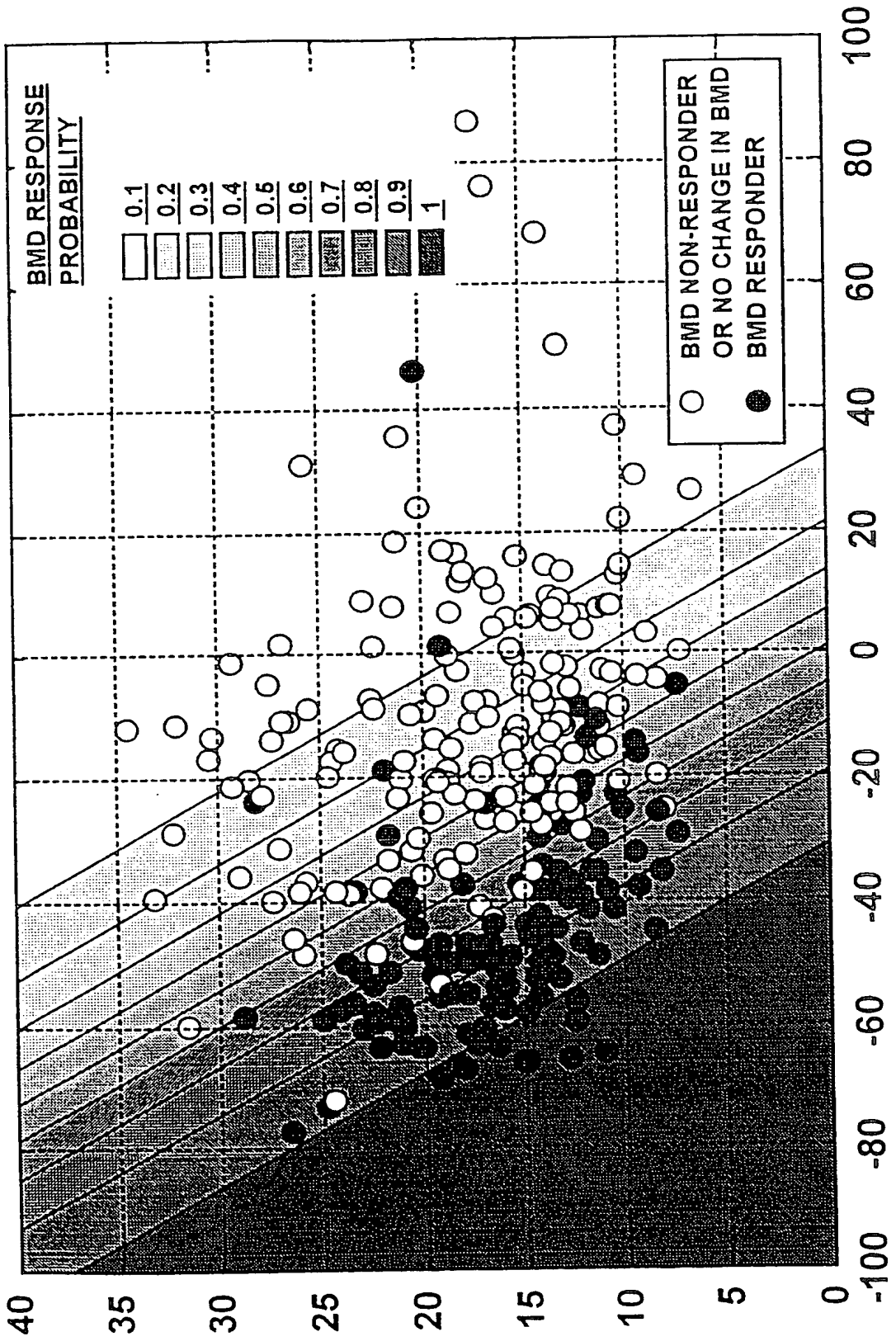
BMD RESPONSE PROBABILITY
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
BMD NON-RESPONDER OR NO CHANGE IN BMD
BMD RESPONDER
BAP AT BASELINE (µg/L)
40
35
30
25
20
15
10
5
0
-100
-80
-60
-40
-20
0
20
40
60
80
100
BAP % CHANGE FROM BASELINE AT 6 MONTHS

Fig. 5

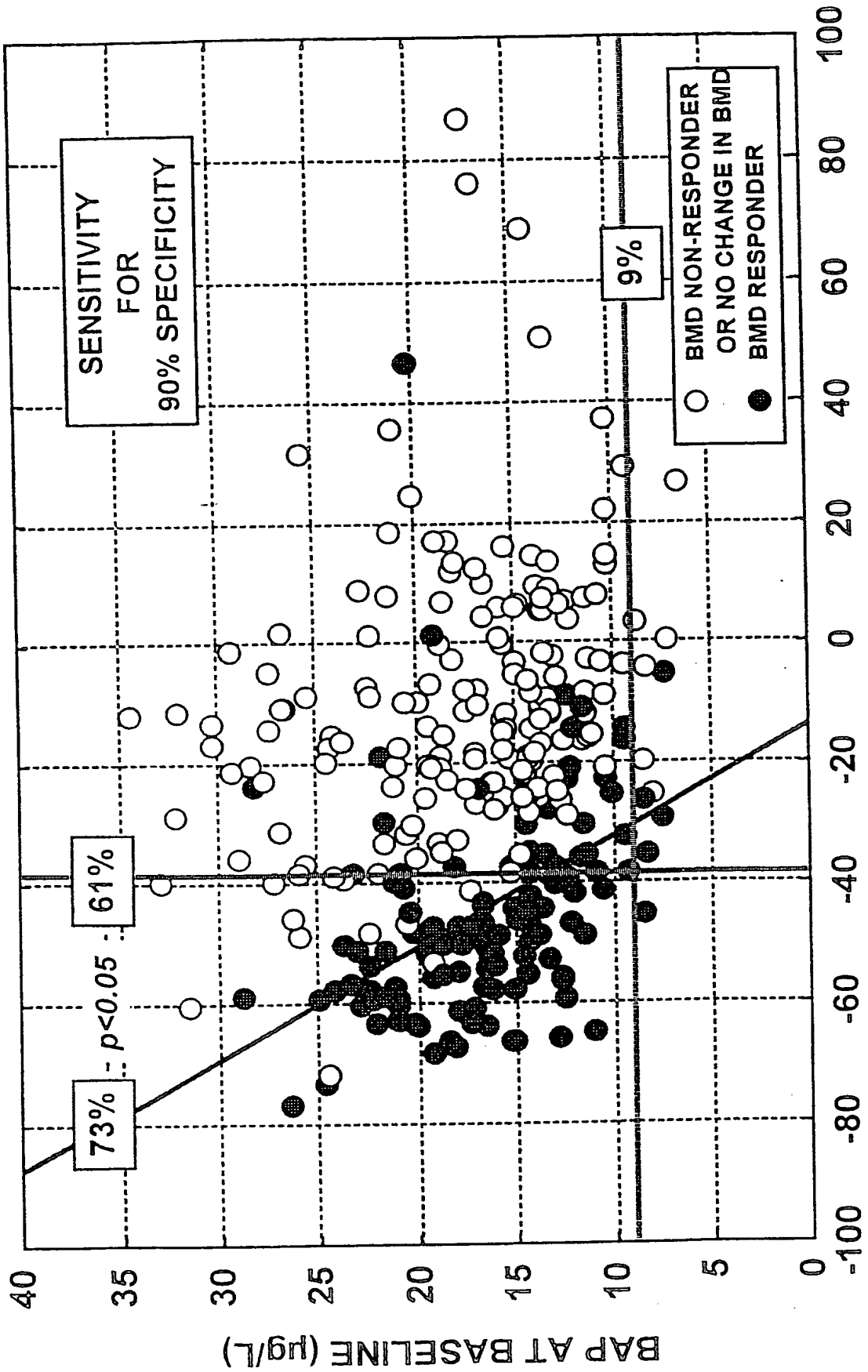
BAP AT BASELINE (µg/L)
40
35
30
25
20
15
10
5
0
BAP % CHANGE FROM BASELINE AT 6 MONTHS
100
80
60
40
20
0
-20
-40
-60
-80
-100
BMD NON-RESPONDER OR NO CHANGE IN BMD
BMD RESPONDER
SENSITIVITY FOR 90% SPECIFICITY
9%
61%
73%
p<0.05

Fig. 6

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/20698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEDLINE, Washington DC USA; abstract no. 97320553, abstract XP002129136 cited in the application & C.J. ROSEN ET AL.: " The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement or calcium supplementation " JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, New York NY USA	1,28
Y	--- -/--	1-42

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
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DOCID: <WO 0022437A1 I >

INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 99/20698

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 124, no. 8, 19 February 1996 (1996-02-19) Columbus, Ohio, US; abstract no. 76765, XP002129138 abstract & P. ALEXANDERSEN ET AL.: " The effect of menopause and hormone replacement therapy on bone alkaline phosphatase" SCAND. J. CLIN. LAB. INVEST. , vol. 55, no. 7, 1995, pages 571-576, Copenhagen DK	1-42
A	BIOLOGICAL ABSTRACTS, Philadelphia PA USA; abstract no. XP002129137 & N. NIELSEN ET AL.: "Estimation of effects of salmon calcitonin in established osteoporosis by biochemical bone markers " CALCIFIED TISSUE INTERNATIONAL, vol. 55, no. 1, 1994, pages 8-11, New York NY USA	1-42

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:DOCID: <WO 0022437A1 I >

CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
G01N 33/68, 33/573

A1

(11) International Publication Number: WO 00/22437

(43) International Publication Date: 20 April 2000 (20.04.00)

(21) International Application Number: PCT/US99/20698

(22) International Filing Date: 9 September 1999 (09.09.99)

(30) Priority Data:
09/172,521 14 October 1998 (14.10.98) US

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(81) Designated States: JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PROGNOSTIC METHOD FOR ANTI–RESORPTIVE TREATMENT

(57) Abstract

The present invention describes a new model based on the logistic combination of the percentage change of the level of a bone marker at a predetermined time period and the level of the bone marker at a baseline or at the predetermined period to predict shortly after initiating an anti–resorptive therapy those patients who will not significantly improve their bone BMD after two years of treatment or those patients who do not comply with therapy. In addition, the present invention also provides for the first time a defined cut–off value that can easily be used in clinical practice to identify individual non–responder or non–compliant patients after a short period of treatment, such as six months.

*(Referred to in PCT Gazette No. 40/2000, Section II)

:DOCID: <WO 0022437A1 IA>

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PROGNOSTIC METHOD FOR ANTI-RESORPTIVE TREATMENT

Background of the Invention

Field of the Invention

The invention relates generally to anti-resorptive treatments of postmenopausal women, and specifically to methods of assessing the long-term efficacy of anti-resorptive treatments in individual postmenopausal women.

Description of the Prior Art

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Osteoporosis is a disease characterized by a low bone mass and architectural deterioration of bone tissue. Osteoporosis leads to increased susceptibility to fracture. Decreased bone mass is one of the main determinants of fracture. Two reasons may cause decreased bone mass: first, an imbalance between bone resorption and bone formation within a remodeling unit due to increased osteoclastic activity and/or decreased osteoblastic activity; second, an increase in the activation frequency, i.e., in the number of remodeling units initiated per unit of time and space. Increased bone turnover resulting from postmenopausal estrogen deficiency is the main determinant of bone loss and can be non-invasively assessed by measuring through serum and/or urine biochemical markers of bone turnover (1,2).

Anti-resorptive therapy, such as estrogen replacement therapy (HRT) and bisphosphonate treatment, have been shown to decrease bone turnover, preventing postmenopausal bone loss and significantly reducing fracture risk both in early and late postmenopausal women (3-6). After two years of anti-resorptive therapy, there is usually a small gain in bone mass in the order of 5% to 10% at the lumbar spine and less than 5% at the femoral neck and forearm. However, the precision error of bone mass measurement of the lumbar spine is about 1% to 2%, even by the most precise techniques, e g., dual or single energy X-ray absorptiometry. Therefore, it is usually necessary to wait up to two years after initiating therapy to determine in a single patient if a treatment is

effective, i.e., increasing bone mass significantly. In most instances, repeating bone mass measurement at a shorter interval may not be helpful for the physician's decision making about compliance and treatment efficacy.

Conversely, anti-resorptive therapy induces a 30% to 60% decrease of markers of resorption and formation that fall within the premenopausal range within only three to six months (7). Thus, several studies have suggested that changes in bone markers after three to six months of treatment could be used to predict changes in bone mineral density (BMD) after one to two years in postmenopausal women treated either with estrogen (7-10) or bisphosphonate (11). In these studies the predictive values of markers was investigated in terms of a correlation between percentage change in markers after three to six months and percentage changes in bone mineral density at one or two years. Reported correlation coefficients are typically of 0.4-0.6, indicating that less than 40% of the inter-individual variance in long-term BMD changes can be predicted from early changes in bone markers. Obviously, this strategy cannot be used to accurately determine the absolute level of bone mass gain in individual patients. However, for the clinician, the primary concern is the fast detection of non-responders, i.e., patients who will fail to demonstrate a significant increase in BMD after one to two years of treatment, either resulting from poor compliance, non-absorption of the drug or no response for unknown reasons. By using marker percentage change from a baseline at six months in a receiving-operating characteristic (ROC) curve analysis model, Rosen et al. (10) recently showed that BMD responders to one year HRT can be predicted with a specificity of 90% but with a sensitivity of only 50%. No such analysis has been reported for bisphosphonate treatment.

Therefore, it is desirable to develop a method useful for predicting bone mass response and compliance of an individual after an anti-resorptive therapy, particularly a bisphosphonate treatment. It is also desirable to develop a method that optimizes the prediction in the early stage of treatment.

Summary of the Invention

It is an object of the present invention to provide a method useful for predicting bone mass response and compliance of an individual undergoing an anti-resorptive therapy. It is also an object of the present invention to develop a method that optimizes predictions in the early stage of the anti-resorptive therapy such that the prediction is specific and sensitive.

These and other objects and advantages are achieved by the methods of the present invention. One aspect of the present invention provides a method for predicting bone mass response and compliance of an individual after an anti-resorptive therapy. In accordance with the present invention, the method basically includes the following steps:

1. measuring a baseline of a bone marker, *i.e.*, serum bone alkaline phosphatase (BAP), in the individual at the beginning of the anti-resorptive therapy;
2. measuring the level of the bone marker in the individual after a predetermined time period of the anti-resorptive therapy, *i.e.*, after six (6) months of therapy; and
3. generating a probability of response in bone mass after a second predetermined period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker either at the baseline or at the first predetermined time period.

According to one embodiment of the present invention, a logistic algorithm based on both the level of bone marker after a first predetermined time period and the change of bone marker level from the baseline is utilized to predict a probability of response in bone mass after a longer predetermined period of therapy, *i.e.*, two (2) years. According to another embodiment of the present invention, a logistic algorithm based on both the level of bone marker at the baseline and the change of bone marker level from the baseline after a predetermined period is utilized to predict a probability of response in bone mass after a longer predetermined period of therapy, *i.e.*, two (2) years.

In implementing the present invention method, a cutoff can be selected by a receiver-operating characteristic (ROC) curve analysis to provide corresponding sensitivity and specificity. The cutoff is a function of the change of the bone marker level at a predetermined time period and the level of bone marker at either the baseline or the predetermined time period. The present invention method utilizes a logistic regression model.

As provided by the present invention, the BMD data may be plotted on a two-dimensional diagram. One dimension of the two-dimensional diagram is bone marker level, and the other dimension the change of bone marker level from the baseline.

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Methods of the present invention provide a number of advantages. As explained in greater detail below, it has been found that methods of the present invention can quickly and accurately identify non-responders from responders to anti-resorptive treatment. For example, it can provide about 72% of sensitivity to predict two-year lumbar spine BMD response or to distinguish placebo from alendronate-treated patient for a given 90% specificity. Therefore, the methods of the present invention provide higher diagnostic specificity and sensitivity than the methods which consider the BAP level and BAP change parameters alone. In addition, by using the methods of the present invention, non-responders (or patients who do not adequately take alendronate) can easily be identified using a simple two-scale graph. In clinical practice, the identification of such patients after a few months of treatment would be useful to insure that alendronate is taken appropriately. Conversely, identification of a positive response to alendronate might improve long-term compliance.

The methods of the present invention are well suited for use during the anti-resorptive treatment for assessing the treatment response and the compliance of the treatment. They may also be applied to other markers of bone formation and bone resorption with different characteristics in terms of reproducibility and responses to alendronate. Furthermore, the methods of the present invention may also be applied to other anti-resorptive therapies such as estrogen replacement therapy. Finally, the methods of the present invention may be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss.

The invention is defined in its fullest scope in the appended claims and is described below in its preferred embodiments.

Description of the Figures

The above-mentioned and other features of this invention and the manner of obtaining them will become more apparent, and will be best understood, by reference to the following description, taken in conjunction with the accompanying drawings. These drawings depict only a typical embodiment of the invention and do not therefore limit its scope. They serve to add specificity and detail, in which:

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FIGURE 1 is a diagram which shows the response of bone alkaline phosphatase to treatment with alendronate (10 mg/day) or placebo in 307 elderly osteoporotic women.

FIGURE 2 is a diagram which shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level after six months of treatment) and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Responders are identified as patients with a percent increase in BMD from baseline after twenty-four months of treatment ≥ 3%. No BMD change was defined as a percent BMD change between –3% and +3%. BMD non-responders were women with a bone loss greater than 3%. The predicted probabilities were computed for differentiating BMD responders from both no BMD change and BMD non-responders considered as a single group.

FIGURES 3A and 3B are diagrams which show the areas under the ROC curve for the prediction of BMD (Fig. 3A) and alendronate-treated patients (vs. placebo, Fig. 3 B) in 307 elderly osteoporotic women. Three predictive models were compared: BAP percent change from baseline, BAP level, and their combination by logistic regression. ROC curves were established for the three discriminants and the areas under the curves computed as a function of the treatment monitoring time. P-values refer to the significance level of the difference between those areas as indicated.

FIGURE 4 is a diagram which shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (after six months of treatment) or their combination by logistic regression in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group.

FIGURE 5 is a diagram which shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level at the baseline and the predicted probability of lumbar BMD positive

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response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women.

FIGURE 6 is a diagram which shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (at the baseline) or their combination by logistic regession in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group.

Detailed Description of the Invention

The present invention is based on the discovery of a new model, based on the logistic combination of the level of bone marker and its percentage change in a patient at six months, to predict soon after initiating alendronate therapy those patients who will not significantly improve their BMD after two years of treatment. The present invention is also based on the discovery of a defined cutoff value that can easily be used in clinical practice to identify individual non-responder patients after only six months of treatment.

Accordingly, one aspect of the present invention provides a method for predicting a treatment response and compliance of an individual after an anti-resorptive therapy. The method comprises the steps of:

a. measuring a baseline of a bone marker in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of the bone marker in the individual after a first predetermined time period of anti-resorptive therapy; and

c. generating a probability of response in bone mass after a second predetermined time period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker at either the baseline or at the first predetermined time period for the purpose of determining the treatment response and compliance.

For the purpose of the present invention, an anti-resorptive therapy is a therapy that decreases bone turnover, prevents bone loss and reduces fracture

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risk. Examples of an anti-resorptive therapy include, but are not limited to, bisphosphonate therapy or estrogen replacement therapy (HRT). In accordance with one embodiment of the present invention, the anti-resorptive therapy is alendronate treatment therapy.

A bone marker may be any biochemical compound that can be used as a mark to reflect any changes in bone formation and bone resorption. Therefore, a bone marker can be any marker of bone formation and bone resorption. In accordance with one embodiment of the present invention, a bone alkaline phosphatase (BAP) is used as a bone marker. BAP is from serum; although, other bone markers from other sources derived from human body parts may also be used, e.g., urine samples.

A level of a bone marker in a patient may be measured by using conventional methods that are known to those skilled in the art. For example, to measure the level of BAP in serum, a two-site immunoradiometric assay using two monoclonal antibodies directed against the human bone isoenzyme may be used. Such a two-site immunoradiometric assay is known to one skilled in the art, and procedures can be obtained from the manufacturer Hybritech Incorporated (San Diego, California). Other known methods for measuring bone markers include high performance liquid chromatography (HPLC), lectin precipitation, heat inactivation and immunoadsorption.

For the purpose of the present invention, the first predetermined time period is a time period within which the decrease of a bone marker has reached a plateau. Such time may vary depending on how fast a bone marker responds to a treatment. For an anti-resorptive therapy, this period may be three to six months. Preferably, for alendronate treatment, a BAP level at the six months treatment is measured. It should be understood that earlier time points may also be valuable, and one skilled in the art can readily determine the time period for measuring a bone marker without undue experimentation in view of the present disclosure.

A response in bone mass may be a change in bone mineral density (BMD). In accordance with one embodiment of the present invention, a change in BMD is used as a response in bone mass for the anti-resorptive therapy. Typically, bone mass from lumbar spine is measured although bone mass from other organs such as, but not limited to, femoral neck and forearm may also be measured.

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A second predetermined time period of the anti-resorptive therapy is a time that a response in bone mass is sufficiently significant so that the responsiveness of the individual undergoing the anti-resorptive therapy can be determined. For example, a BMD change at two years of anti-resorptive therapy may be used to indicate whether a patient is a responder to the treatment. It will be understood that different time points may also be valuable. Therefore, a responder is defined as an individual, for example a woman, demonstrating a BMD increase after two years of treatment of 3% or more. A BMD change between -3% and +3% is considered as no significant change and an individual with a bone loss greater than 3% is considered as a non-responder.

A probability of a response in bone mass after a second predetermined time period of the anti-resorptive therapy may be generated by a logistic algorithm, preferably, a logistic regression algorithm, based on a change of the bone marker level from the baseline at a first predetermined period and the level of bone marker at either the baseline or the first predetermined time period. A logistic algorithm may be used to compute the statistical significance levels of each parameter estimated in the logistic equation. A logistic algorithm model may be evaluated based on the maximum likelihood estimation and Chi-square tests. A logistic regression model is appropriate only when the predicted probability ((p)-level) associated with a Chi-square and the slopes of each variable in the logistic equation are statistically significant (for example, $p<0.05$). Such a predicted probability from the logistic regression may be used to distinguish positivity (i.e., the BMD response of a patient in an alendronate group) from negativity.

In accordance with one embodiment of the present invention, a cutoff may be selected to provide a corresponding sensitivity and specificity of the prediction of the present invention. In one embodiment, a cutoff may be established by a receiver-operating characteristic (ROC) curve analysis. In this embodiment, the cutoff (t) is a function of two variables, i.e., BAP percentage change from baseline and BAP level at either the baseline or at six months, according to the following logistic equation:

$$t = \frac{1}{1 + e^{-Z}} \qquad [1]$$

where

$$Z = a + b \times (\text{change}) + c \times (\text{level}) \qquad [2]$$

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and a, b and c are logistic regression parameter estimates. Equation [1] can be transformed as:

$$\text{level} = -\frac{a - \log\frac{t}{1-t}}{c} - \frac{b}{c} \times (\text{change}) \quad [3]$$

Equation [3] indicates that for any particular t value, the cutoff corresponds to a straight line when patient's bone marker data is reported in two-dimension scatter-plots where axes represent the two variables, i.e., BAP percentage change from baseline (X) and absolute BAP level at either baseline or at six months (Y). This straight line separates positive from negative data points with a sensitivity and specificity that are set when the cutoff value t is selected by ROC curve analysis.

For example, according to one embodiment of the present invention, the individual patient's BMD data can be scatter-plotted on a two-dimensional diagram, where one dimension is serum BAP level at six months, and the other is percentage change of serum BAP level at six months. For a particular cutoff value, it corresponds to a straight line on the diagram, which separates the responsive from non-responsive points with a sensitivity and specificity set when the cutoff value is selected by ROC curve analysis. Likewise, in accordance with another embodiment of the present invention, the individual patient's BMD data may be scatter-plotted on a two-dimensional diagram where one dimension is serum BAP level at the baseline and the other is percentage change of serum BAP level at six months. Here, the "responsive" or BMD "response" is defined as an increase of the BMD by three percent (3%) or more over the long-term, *i.e.*, two (2) years of the anti-resorptive therapy.

Therefore, described generally, another aspect of the present invention provides a method of predicting a treatment response of an individual after a therapy. The present invention method comprises the steps of:

a. measuring the baseline of a first variable comprising a biochemical marker in the individual at the beginning of therapy;

b. monitoring the level of the first variable in the individual undergoing the therapy over a second variable; and

c. deriving a probability of treatment response from at least the first order derivative of the first variable over the second variable and

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the first variable either at the baseline or at a time determined by the second variable.

One type of therapy is anti-resorptive therapy. An example of the biochemical marker is serum bone alkaline phosphatase (BAP). The second variable may be time duration or other varying factors such as dosage of medicine, *etc.* When the second variable is time, the first order derivative of the serum BAP level is simply the change of the serum BAP level over a period of time. In accordance with one embodiment, the first variable may be the BAP level at the baseline. In accordance with another embodiment of the present invention, the first variable may be the BAP level at the time the change is determined.

The present invention provides a new model using the logistic combination of both the actual value and the percentage change of a bone marker after a short-term treatment period to identify patients who will subsequently demonstrate a positive bone mass response. This model provides a higher diagnostic specificity and sensitivity than the two individual parameters used alone. Using this model, non-responders (or patients who do not comply with the treatment) can easily be identified using a simple two-scale graph.

The present invention also employs a cutoff based on a logistic regression model, including both percentage change of a bone marker from the baseline level and the bone marker level at a predetermined time. This combination allows a substantial increase in the sensitivity of predicting a positive bone mass response with a similar specificity when the two parameters are used alone.

The methods of the present invention are well suited for use during the anti-resorptive treatment for assessing the treatment response and the compliance of the treatment. They may be applied to different markers of bone formation and bone resorption with different characteristics in terms of reproducibility and responses to treatment. Furthermore, the methods of the present invention may also be applied to a variety of anti-resorptive therapies, such as estrogen or alendronate treatment. Finally, the methods of the present invention may be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss (1, 15, 16).

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The following examples illustrate one detailed implementation of the present invention method.

EXAMPLES

Subjects

Three hundred and seven (307) women, aged 45-78 years (mean age: 64.0±7 years), were studied. They were all at least five years past a natural menopause (mean 17.6±8.0 years), and had lumbar spine BMD measured by dual energy X-ray absorptiometry (DXA) more than 2.5 standard deviation (SD) below the normal mean for premenopausal women. These late-postmenopausal osteoporotic women were enrolled in a two-year, double-blind, placebo-controlled trial, where the bisphosphonate alendronate (4-amino-l-hydroxybutylidene-1,1-bisphosphonate, Merck, USA) was administered orally once daily in the morning. Analysis was restricted to patients on placebo and to those treated with 10mg/day alendronate, i.e., the dose which is approved for the treatment of postmenopausal osteoporosis. All subjects also received 500 mg/day of elemental calcium (as carbonate).

Blood samples for biochemical marker measurements were obtained at the baseline, three, six, twelve and twenty-four months after initiation of therapy. They were stored at -20 C until assayed. All samples collected from a single subject throughout the study period were measured in a single assay run. Lumbar spine BMD was determined by DXA at the baseline and at twenty-four months of treatment, using a Hologic QDR-1000 densitometer (Hologic, Waltham, USA). The change of BMD with time was expressed in percentage change from the baseline. The study was approved by the ethics committees of the participating medical centers.

Measurement of Serum Bone Alkaline Phosphatase (BAP)

Serum BAP was measured with a human specific two-site immunoradiometric assay using two monoclonal antibodies directed against the human bone isoenzyme. BAP purified from human SAOS-2 osteosarcoma cells was used as a standard (Ostase®, Hybritech Incorporated, San Diego, California). In this assay, monoclonal antibodies cross-react by only 16% with the circulating liver isoenzyme. The sensitivity of the assay is 0.2 ng/ml, and the

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intra and inter assay coefficient of variation (CV) are less than 7% and 9%, respectively (12). The premenopausal range was established in one hundred thirty-four (134) healthy premenopausal (mean age: 41±5 years) women, belonging to a prospective population-based cohort (OFELY study: 1039 healthy volunteers, 31-89 years). The premenopausal range was 8.7±2.7 mg/L (2).

Statistical Analysis

Long-term variability of BAP measurement within a patient was assessed by the within-subject coefficient of variation on the five samples collected from the placebo group (N=175) over a twenty-four months period. The changes in biochemical markers of bone turnover with time under the treatment with alendronate and placebo were evaluated by analysis of variance. The correlation coefficients between the percentage change in lumbar spine BMD at month 24, absolute level of BAP, and the percentage change of BAP at month 6 were assessed by simple and multiple regression analysis.

Logistic Regression Model to Predict Long-term BMD Response and Compliance by BAP Level at Either Baseline or Six Months and Percentage BAP Change at 6 Months. Logistic regression was used to compute the probability of each patient to be in the alendronate-treated group or to predict their spine BMD response as a function of BAP percentage change from baseline and BAP level at three, six, twelve or twenty-four months of the treatment. Response to therapy was defined according to the percentage change from baseline in spine BMD after twenty-four months. As this variable is continuous, it has been re-coded as a binary variable (response vs. non-response) for logistic regression. Given the precision error CV of bone mass measurement by DXA, i.e., around 1%, a change in BMD would be significant at the individual level if it exceeds 1.96×(square root of 2)×CV, i.e., 2.8%. Thus, responders were defined as women demonstrating a percentage BMD change of 3% or more after two years. Non-responders to treatment were considered as patients with a two-year BMD change lower than 3%. Maximum likelihood estimation and Chi-square tests were used to estimate the goodness of fit of the overall model, and the statistical significance levels of each parameter estimated in the logistic equation were computed. The logistic regression model was considered appropriate only when the probability (p)-levels associated with Chi-square and the slopes of each variable in the logistic equation were statistically significant ($p<0.05$). Predicted probabilities (p) from the logistic regression were used to distinguish positivity (BMD response or patient in the alendronate group) from negativity (non-BMD

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response or patient in the placebo group). Cutoffs which provide appropriate sensitivity and specificity were established by ROC curve analysis.

In this model the cutoff t is a function of two variables, i.e., BAP percentage change from baseline and BAP level, according to the following classical logistic equation:

$$t = \frac{1}{1 + e^{-Z}} \qquad [1]$$

where

$$Z = a + b \times (\text{change}) + c \times (\text{level}) \qquad [2]$$

and a, b and c are logistic regression parameter estimates. Equation [1] can be transformed as:

$$\text{level} = -\frac{a - \log\frac{t}{1-t}}{c} - \frac{b}{c} \times (\text{change}) \qquad [3]$$

Equation [3] indicates that for any particular t value, the cutoff corresponds to a straight line when patients' bone marker date is reported in two-dimension scatter-plots where axes represent the two variables, BAP percentage change from baseline (X) and absolute BAP level at either the baseline or at six months (Y). This straight line separates positive from negative data points with a sensitivity and specificity that are set when the cutoff value t is selected by ROC curve analysis.

Comparison of the Different Models of Prediction. The model based on logistic regression analysis which combines both BAP level at either the baseline or six months and BAP percentage change at six months was compared with models using either one of these two individual discriminants. Overall discriminant performances were compared in terms of area under the ROC curve. Comparisons were also performed for a given threshold of specificity by paired Chi-square tests.

RESULTS

Effect of Alendronate Treatment on BAP Levels and Relationships with BMD Changes.

At baseline, BAP levels were increased by a mean 95% compared to premenopausal values and 68.4% of patients had levels above the upper limit of

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premenopausal range (mean+2SD: 14.1mg/L) (data not shown). Upon alendronate treatment, BAP showed a progressive decrease reaching a nadir after six months of treatment (-44%), and the levels did not further change for the duration of the study (Fig. 1). Figure 1 shows a response of bone alkaline phosphatase to treatment with alendronate (10 mg/day) or placebo in 307 elderly osteoporotic women. Data are the mean±1 SEM. After six months of alendronate treatment, 95% of values were within the premenopausal range.

The percentage change from baseline in spine BMD at twenty-four months correlated significantly with both BAP level at six months and the percentage change BAP at six months ($r = -0.61$ and -0.60, $p < 0.001$, respectively).

Combination of Level and Percentage Change of BAP at 6 Months to Monitor Alendronate Treatment

Based on the percentage change in spine BMD after two years of treatment (alendronate and placebo), 45% of women were classified as BMD responders (increase in BMD ≥3%), 39.7% as no BMD change (BMD change -3% to +3%), and 15.35 as non-responders (loss of BMD >3%). Figure 2 shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP level after six months of treatment, and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Responders are identified as patients with a percent change in lumbar BMD from baseline after twenty-four months of treatment at or greater than 3%. The two latter groups, (i.e., no BMD change and non-responders) have a similar distribution of BAP levels at six months and BAP percentage change at six months, and could not be discriminated by these two parameters. Thus, these two groups were combined in the subsequent analyses, and the value of BAP levels, BAP percentage change at six months and the logistic combination of these two parameters were investigated to discriminate BMD responders (increase in BMD ≥3%).

Figure 5 shows the relationship between the percentage change from the baseline after six months of treatment of BAP, BAP levels at the baseline, and the predicted probability of lumbar BMD positive response (after twenty-four months of treatment) as computed by logistic regression in 307 elderly osteoporotic women. Likewise, responders are identified as patients with a percent change in lumbar BMD from baseline after twenty-four months of

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treatment at or greater than 3%. As shown in Figure 5, the two latter groups, (i.e., no BMD change and non-responders) have a similar distribution of BAP levels at the baseline and BAP percentage change at six months, and could not be discriminated by these two parameters. Thus, these two groups were combined in the subsequent analyses, and the value of BAP levels at the baseline, BAP percentage change at six months and the logistic combination of these two parameters were investigated to discriminate BMD responders (increase in BMD $\geq$ 3%).

Levels of BAP at either the baseline or at six months and percentage changes of BAP at six months were significant and independent predictors of BMD response at two years (i.e., with a BMD gain at two years of 3%, or more) in logistic regression analysis ($p < 0.02$ for both parameters). These two parameters were thus combined to compute the predicted probability of individual patients to respond to alendronate treatment (see statistical analysis Figure 2 and Figure 5). As expected, responders and non-responders have distinct distributions in relation to their predicted probabilities, the former group being characterized by higher probabilities of spine BMD gain at two years.

The area under the ROC curve was used to compare the discriminative power of this combination model with those using only BAP level at six months or percentage BAP change at six months. Figure 3 shows the area under the ROC curve for the prediction of BMD (Panel A) and alendronate-treated patients (vs. placebo, panel B) in 307 elderly osteoporotic women. Three predictive models were compared: BAP percent change from baseline, BAP level, and their combination by logistic regression. ROC curves were established for the three discriminants and the areas under the curves computed as a function of the treatment monitoring time. P-values refer to the significance level of the difference between those areas as indicated. As shown in Figure 3, the discrimination between BMD responders/non-responders (Fig. 3A) and alendronate-treated patients/placebo (Fig. 3B) increases with time after initiating therapy, a plateau being reached at six months for the prediction of BMD response. BAP percentage change from the baseline and the BAP level appear to be equivalent when used separately, except at three months when BAP level shows the lowest discrimination power. Irrespective of monitoring time, the discrimination power provided by logistic combination of BAP percentage change from baseline and BAP level is superior to that obtained with either of the two monitoring parameters taken separately.

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A cutoff can be selected by logistic regression and ROC curve analysis (Figs. 4 and 6) to provide after six months of treatment a specificity of 90%. Figure 4 shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (from baseline after six months of treatment) or BAP level (after six months of treatment) or their combination by logistic regression in 307 elderly osteoporotic women. Discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group. This cutoff implies that no more than 10% of women classified with markers as having a subsequent positive BMD response (i.e., an increase at two years > 3%) would be false positive. This cutoff results in a 72% sensitivity in the detection of patients who will demonstrate a favorable spine BMD increase after two years of treatment.

Figure 6 shows the prediction of lumbar spine BMD response to twenty-four months of treatment, based on either BAP percent change (form baseline after six months of treatment) or BAP level (at the baseline) or their combination by logistic regression in 307 elderly osteoporotic women. Likewise, discriminant cutoff values were selected to provide 90% specificity for non-response prediction for each model, so that resulting sensitivities for positive response prediction could be compared. BMD responders, no BMD change and BMD non-responders were defined as in Figure 2. Sensitivity and specificity figures apply for the discrimination of BMD responders from BMD non-responders and no BMD change considered as a single group. This cutoff implies that no more than 10% of women classified with markers as having a subsequent positive BMD response (*i.e.*, an increase at two years > 3%) would be false positive. This cutoff results in a 73% sensitivity in the detection of patients who will demonstrate a favorable spine BMD increase after two years of treatment.

By comparison, BAP percentage change from baseline, BAP level at six months or BAP level at the baseline taken separately would provide significantly lower sensitivities for the same 90% specificity (Table 1 and Figure 6). Table 1 shows the sensitivity of different models to predict two-year lumbar spine BMD response or to distinguish placebo from alendronate-treated patients for a given

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90% specificity in each model. When patients with no significant BMD change (-3% < %BMD change < +3%) were excluded from the analysis, for the same 90% specificity, the sensitivity to detect responders increased slightly for each model and was also highest for the logistic regression combination (67%, 70%, and 75% for BAP level at six months, percentage change of BAP at six months and the logistic combination of BAP level and % BAP change, respectively) (data not shown). A cutoff can also be set to differentiate women on placebo from alendronate-treated patients by logistic regression and again the sensitivity obtained from the combination of BAP level and BAP percentage change at six months was higher than that obtained by each parameter alone (Table 1).

TABLE 1

Predictive model	BMD response		Alendronate/placebo	
	Cutoff	Sensitivity (%)	Cutoff	Sensitivity (%)
BAP level at 6 months	9.5µg/L	59V	.94 µg/L	66
% BAP change at 6 months	-38.2%	61	-38.5	71
BAP level at 6 months + % BAP change at 6 months	BAP level=1.7-0.28* % BAP change	72*	BAP level = 3.5-0.24* % BAP change	82*

Combination of BAP Percentage Change and BAP Level at 6 Months Compared to BAP Least Significant Change Cutoff for Treatment Monitoring

The long-term within patient variability of BAP levels over a twenty-four-month period in the placebo group (n=175) was 15.7%, resulting in a least significant change cutoff of 43.5%. When patients with BAP percentage change from baseline lower than –43.5% at six months of treatment are classified as BMD responders, BMD response can be predicted with a 93% specificity which is comparable to the 90% of the logistic model response at two years but with

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only 54% of sensitivity, which is significantly lower than the 72% obtained from the logistic combination ($p < 0.002$). Similarly, the least significant change cutoff is less powerful to differentiate placebo from alendronate- treated patients than the combination model with a 22% lower sensitivity (60% vs. 82%, $p<0.001$) for a similar specificity (95% vs. 90%, NS).

It might be argued that BAP least significant change calculated from the placebo group overestimates BAP variability in normal individuals. A value of 25% has been proposed as a cutoff of significant change of BAP (13). In this study, a cut-point of -25% BAP percentage change from baseline at six months would result in sensitivities for treated individuals or for positive BMD response prediction statistically similar to those observed with the logistic (85% vs. 82% and 78% vs. 72%, respectively). In both cases, however, resulting specificity would be much lower than that obtained from the logistic combination: 78% and 75% versus 90%, respectively ($p<0.001$).

DISCUSSION

The present invention provides a new model using the logistic combination of both an absolute level and the percentage change of a bone marker after a short-term treatment period to rapidly identify patients who will subsequently demonstrate positive BMD responses. This model gives higher diagnostic specificity and sensitivity than the two individual parameters considered alone. In addition, using this model, non-responders (or patients who do not adequately take alendronate) can easily be identified using a simple two-scale graph.

The present invention is based on the discovery that the combination of both the absolute level of BAP and the percentage change of BAP at the six-month date of an anti-resorptive therapy can be used to predict the probability of a positive BMD response after two years of the therapy, which responses is determinative of the effectiveness of the treatment. It is a discovery of the present invention that as the decrease in BAP reaches a plateau after six months of treatment, this six-month time point is the most adequate to test the usefulness of bone markers to predict bone mass response, although earlier time points may also be valuable (9-11). It is an observation of the present invention that there is a significant correlation between the percentage change of bone marker levels at six months and the percentage change of spine BMD at two years with a correlation coefficient slightly lower than that previously

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observed in a smaller study (n=75) with the same drug (-0.62 vs. -0.77). In addition, a strong negative correlation between absolute levels of bone markers at six months and percentage spine BMD changes at six months is also surprisingly observed, which suggests that this parameter could also be used to monitor the efficacy of alendronate treatment. Based on these discoveries and observations, the present invention combines these two parameters, i.e., percentage BAP change and BAP level, to improve the prediction of bone mass response.

In addition, it is another discovery of the present invention that the combination of both the absolute level of BAP at the baseline and the percentage change of BAP at the six-month date of an anti-resorptive therapy can be used to predict the probability of a positive BMD response after two years of the therapy. Based on this discovery, the present invention combines these two parameters, *i.e.*, BAP level at the baseline and percentage BAP change at six months to improve the prediction of bone mass response.

Using a cutoff based only on the percentage change from baseline of bone marker levels after thee to six months of treatment, i.e., when the decrease of bone turnover has reached a plateau, may not be sufficient to accurately identify patients who will respond favorably to treatment in terms of BMD gain after two years. Indeed, it seems reasonable to consider that patients who have relatively low bone marker levels before treatment may only demonstrate a slight decrease in bone marker and might not be identified as responders despite levels at six months within the normal range, demonstrating the efficacy of the treatment to normalize bone turnover. Therefore, a parameter which represents the level of bone turnover at either the baseline or at a level reached after treatment might be an additional and independent predictor of BMD response.

Based on these beliefs and observations, the present invention performed logistic regression, including both percentage change from baseline and BAP level at either the baseline or at six months to predict BMD responders. It is found that these two parameters are significant and independent predictors of BMD response and their combination provides a significantly higher predictive value than each parameter alone. For example, a high specificity, i.e., the proportion of non-responders who are identified by the predictive model at six months and who indeed did not demonstrate a significant gain in spine BMD at two years, is likely to be the most relevant option that could lead to therapeutic adjustment, including changing drug or dose. For a given 90% specificity, it is

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found that combining the percentage change and BAP level at six months, one could improve significantly the sensitivity by 11% to 13%, compared to using either of the two parameters alone. In addition, the predictive performance of the logistic model was compared with a cutoff based on the least significant change of BAP, which has been suggested as an adequate means tool to detect responders. Based on the five measurements performed on the 175 women of the placebo group, we estimated the least significant change as 43.5%, which is similar to the mean decrease in BAP after six months of alendronate treatment. Such a high least significant change calculated from the placebo group may overestimate BAP variability in normal individuals, because of calcium supplementation in placebos which is likely to decrease bone turnover. Therefore, a -25% cutoff was also used, which has been previously suggested as a more realistic least significant change for that marker (13). It was found that whatever the cutoff of least significant changes considered, the logistic combination model gave significantly higher predictive performance for BMD response or for differentiating placebo from alendronate-treated patients.

This model may be generalizable to other populations. Although this model was constructed from predicted probabilities which are as any predictive value dependent on the prevalence of positive cases in the study population used for their computation by logistic regression, the deduced cutoff lines used to differentiate between positive (BMD responder) and negative (non-responder) cases with defined sensitivity and specificity characteristics are independent of any prevalence. Thus, these cutoff lines determined in this large population are likely to be useful to provide corresponding sensitivity and specificity values in any population with comparable properties in terms of BAP response, even if expected prevalence of positive cases are different. It could also be argued that this predictive model may be more difficult to use than a single percentage BAP change parameter because it requires combining two parameters. However, as illustrated in Figures 4 and 6, non-responders can easily be distinguished from responders by reporting patient characteristics (% BAP change at six months and BAP level at six months or at the baseline) in a two-scale graph with the cutoff as a straight line.

This model was developed using samples from a placebo-controlled clinical trial and it is hypothesized that the biochemical changes observed in placebo-treated women would reflect changes observed in non-compliant patients. In clinical practice, the identification of such patients after a few

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months of treatment would be useful to insure that alendronate is taken appropriately. Conversely, identification of a positive response to alendronate might improve long-term compliance.

This model may be applicable to other markers of bone formation and bone resorption with different characteristics in terms of reproducibility and response and to other anti-resorptive therapies such as estrogen. This model could also be used to test if the combination of different markers, such as one of formation and one of resorption, could improve the predictive performance as it has been suggested for the estimation of the spontaneous rate of bone loss (14, 15).

The foregoing is meant to illustrate, but not to limit, the scope of the invention. Indeed, those of ordinary skill in the art can readily envision and produce further embodiments, based on the teachings herein, without undue experimentation.

The present invention may be embodied in other specific forms without departing from its essential characteristics. The described embodiment is to be considered in all respects only as illustrative and not as restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of the equivalence of the claims are to be embraced within their scope.

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What is claimed is:

1. A method for predicting a treatment response and compliance of an individual after an anti-resorptive therapy, comprising the steps of:

a. measuring a baseline of a bone marker in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of the bone marker in the individual after a first predetermined time period of anti-resorptive therapy; and

c. generating a probability of response in bone mass after a second predetemrined time period of the anti-resorptive therapy from a change of the bone marker level from the baseline and the level of bone marker at either the baseline or at the first predetermined time for the purpose of determining the treatment response and compliance.

2. The method of claim 2, wherein the probability is generated by using a logistic algorithm.

3. The method of claim 2, wherein the logistic algorithm is a logistic regression algorithm.

4. The method of claim 1, wherein the bone marker is selected from a group consisting of markers of bone formation, and marks of bone resorption.

5. The method of claim 4, wherein the bone marker is serum bone alkaline phosphonate.

6. The method of claim 1, wherein the anti-resorptive therapy is bisphosphonate treatment therapy or estrogen replacement therapy.

7. The method of claim 6, wherein the anti-resorptive therapy is alendronate treatment.

8. The method of claim 1, wherein the first predetermined time period is about 3 to 6 months.

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9. The method of claim 8, wherein the first predetermined time period is about 6 month of anti-resorptive therapy.

10. The method of claim 1, wherein the second predetermined time period is about two years of the anti-resorptive theray.

11. The method of claim 1, further comprising a step of selecting a cutoff to provide corresponding sensitivity and specificity.

12. The method of claim 11, wherein said cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

13. The method of claim 11, wherein said cutoff is a function of the change of bone marker level and the level of bone marker at either the baseline or at the first predetermined time.

14. The method of claim 1, further comprising the steps of plotting the bone mass of the individual on a two-dimensional diagram.

15. The method of claim 14, wherein one dimension of the two-dimensional diagram is the bone marker level, and the other dimension of the two-dimensional diagram is the change of bone marker level.

16. The method of claim 1, wherein the bone mass is measured by bone mineral density (BMD).

17. The method of claim 16, wherein the response is defined as a BMD increase of three percent (3%) or more over the second predetermined time period of the anti-resorptive therapy.

18. A method of predicting lumber spine bone mineral density (BMD) response and compliance of an individual after an anti-resorptive therapy, comprising the steps of:

a. measuring a baseline of serum bone alkaline phosphatase (BAP) in the individual at the beginning of the anti-resorptive therapy;

b. measuring a level of serum BAP in the individual after a first predetermined time period of the anti-resorptive therapy;

c. calculating a percentage change of serum BAP level from the baseline; and

d. determining a probability of a response in lumber spine BMD after a second predetermined time period of the anti-resorptive therapy by utilizing a logistic regression algorithm based on the percentage change of serum BAP level and the level of serum BAP at either the baseline or the first predetermined time.

19. The method of claim 18, further comprising a step of selecting a cutoff to provide a corresponding sensitivity and specificity.

20. The method of claim 19, wherein the cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

21. The method of claim 19, wherein the cutoff is a function of the percentage change of serum BAP level and the level of serum BAP at either the baseline or the first predetermined time.

22. The method of claim 18, wherein the algorithm employs logistic regression parameter estimates.

23. The method of claim 18, further comprising the step of plotting the lumber spine BMD of the individual on a two-dimensional diagram.

24. The method of claim 23, wherein one dimension of the two-dimensional diagram is the serum BAP level, and the other dimension of said two-dimensional diagram is the percentage change of the serum BAP level.

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25. The method of claim 18, wherein the first predetermined time period of the anti-resorptive therapy is six (6) months.

26. The method of claim 18, wherein the second predetermined time period of the anti-resorptive therapy is two (2) years.

27. The method as of claim 18, wherein the response is defined as an increase of the lumber spine BMD by three percent (3%) or more over the second predetermined time period of the anti-resorptive therapy.

28. A method of predicting a treatment response of an individual after a therapy, comprising the steps of:

a. measuring the baseline of a first variable comprising a biochemical marker in the individual at the beginning of the therapy;

b. monitoring the level of the first variable in the individual undergoing the therapy over a second variable; and

c. deriving a probability of treatment response from at least the first order derivative of the first variable over the second variable and the first variable either at the baseline or at a time determined by the second variable.

29. The method of claim 28, wherein the biochemical marker is serum bone alkaline phosphatase (BAP).

30. The method of claim 28, wherein the second variable is time.

31. The method of claim 30, wherein the at least the first order derivative of the first variable over the second variable is the change of the level of the first variable over a period of time.

32. The method of claim 31, wherein the period of time is six (6) months.

33. The method of claim 31, wherein the probability of a treatment response is predicted for a longer period of time.

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34. The method of claim 33, wherein the longer period of time is two (2) years.

35. The method of claim 28, wherein said treatment response is measured by a bone mineral density (BMD) response.

36. The method of claim 35, wherein said response is defined as a BMD increase of three percent (3%) or more over said long-term of said anti-resorptive therapy.

37. The method of claim 28, wherein said probability of treatment response is derived by utilizing a logistic regression algorithm.

38. The method of claim 37, further comprising a step of selecting a cutoff to provide corresponding sensitivity and specificity.

39. The method of claim 38, wherein said cutoff is selected by a receiver-operating characteristic (ROC) curve analysis.

40. The method of claim 38, wherein said cutoff is a function of at least the first order derivative of said first variable over said second variable and the first variable either at the baseline or at a time determined by the second variable.

41. The method of claim 28, further comprising the steps of plotting said treatment response of said individual on a two-dimensional diagram.

42. The method of claim 41, wherein one dimension of said two-dimensional diagram is said first variable, and the other dimension of said two-dimensional diagram is said at least the first order derivative of said first variable over said second variable.

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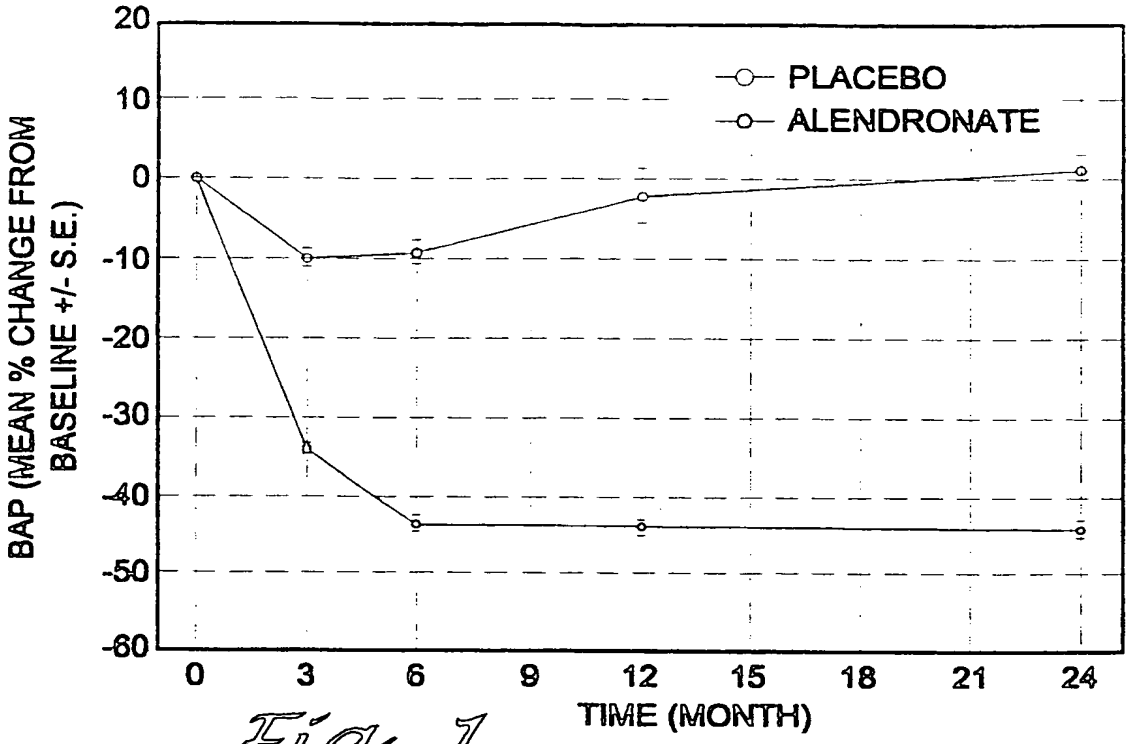
PLACEBO
ALENDRONATE
BAP (MEAN % CHANGE FROM BASELINE +/- S.E.)
20
10
0
-10
-20
-30
-40
-50
-60
0
3
6
9
12
15
18
21
24
TIME (MONTH)

Fig. 1

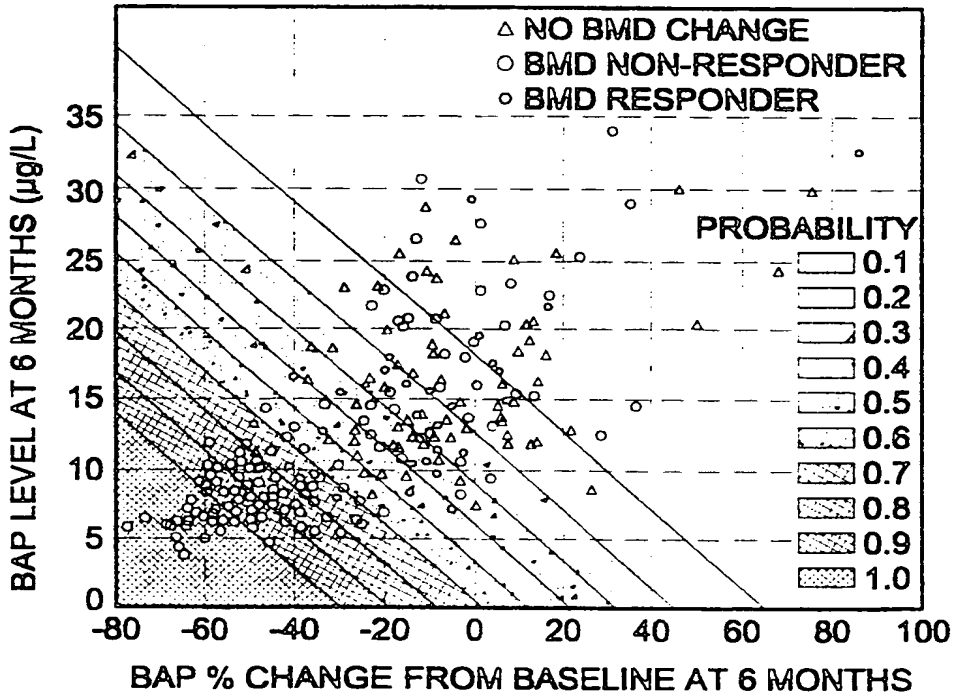
NO BMD CHANGE
BMD NON-RESPONDER
BMD RESPONDER
PROBABILITY
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
BAP LEVEL AT 6 MONTHS (µg/L)
35
30
25
20
15
10
5
0
-80
-60
-40
-20
0
20
40
60
80
100
BAP % CHANGE FROM BASELINE AT 6 MONTHS

Fig. 2

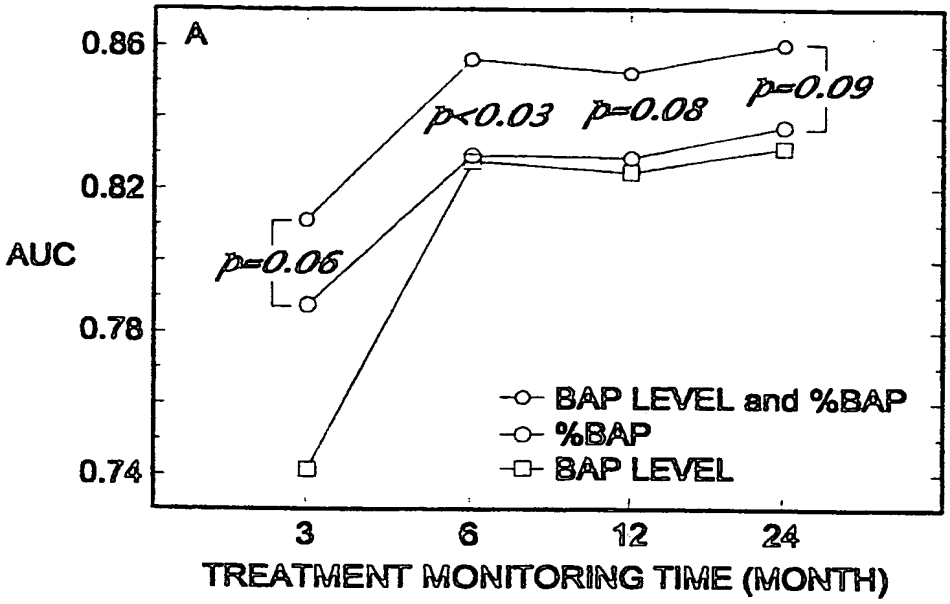
A
0.86
0.82
0.78
0.74
AUC
p=0.06
p<0.03
p=0.08
p=0.09
BAP LEVEL and %BAP
%BAP
BAP LEVEL
3
6
12
24
TREATMENT MONITORING TIME (MONTH)

Fig. 3A

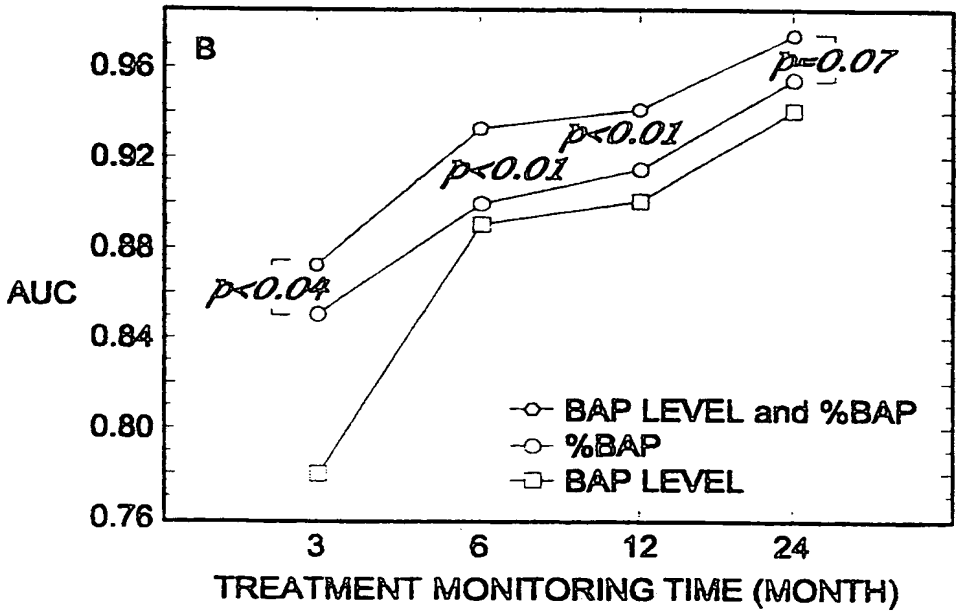
B
0.96
0.92
0.88
0.84
0.80
0.76
AUC
p<0.04
p<0.01
p<0.01
p=0.07
BAP LEVEL and %BAP
%BAP
BAP LEVEL
3
6
12
24
TREATMENT MONITORING TIME (MONTH)

Fig. 3B

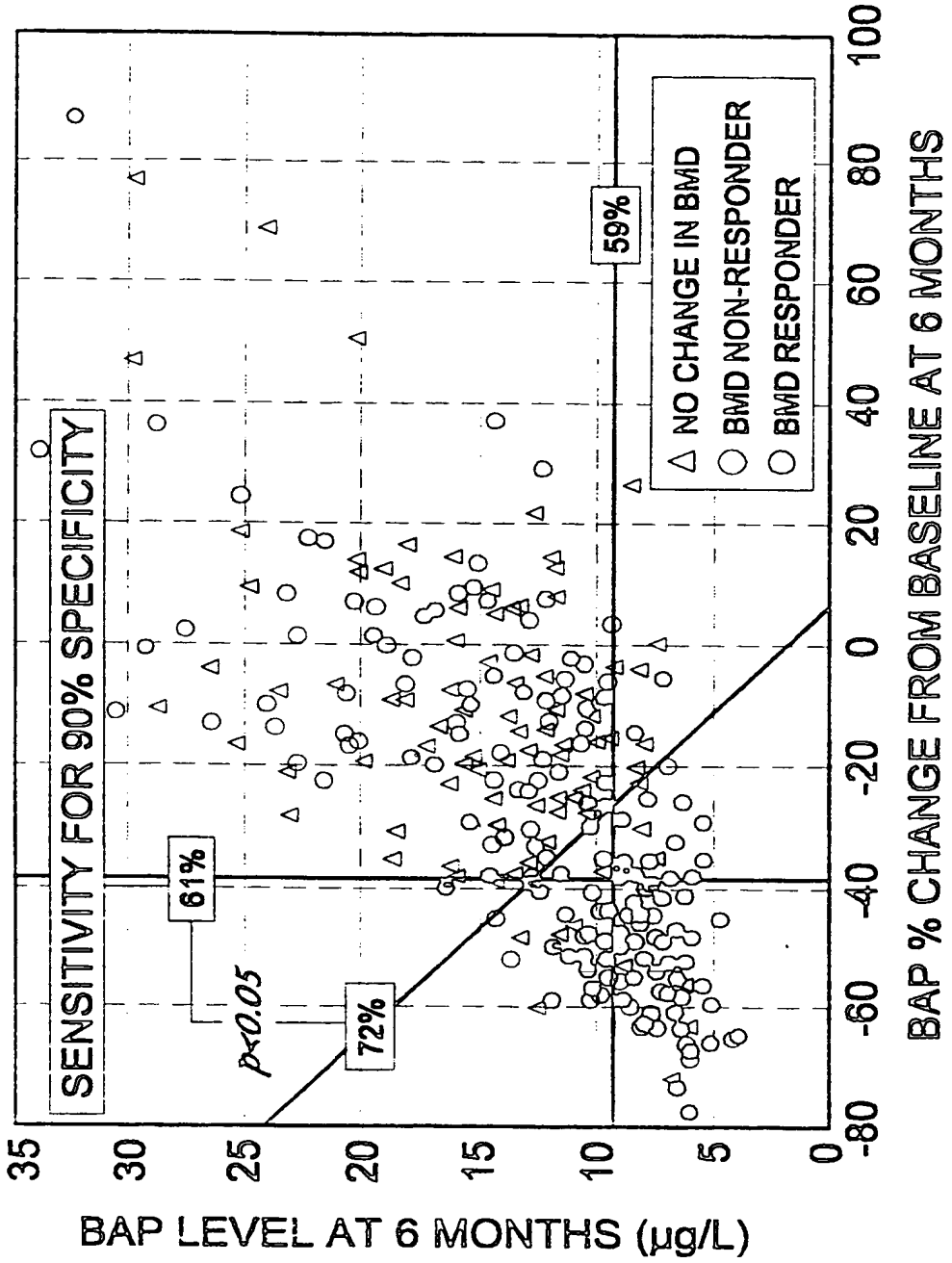

Fig. 4

DOCID: <WO 0022437A1 IA>

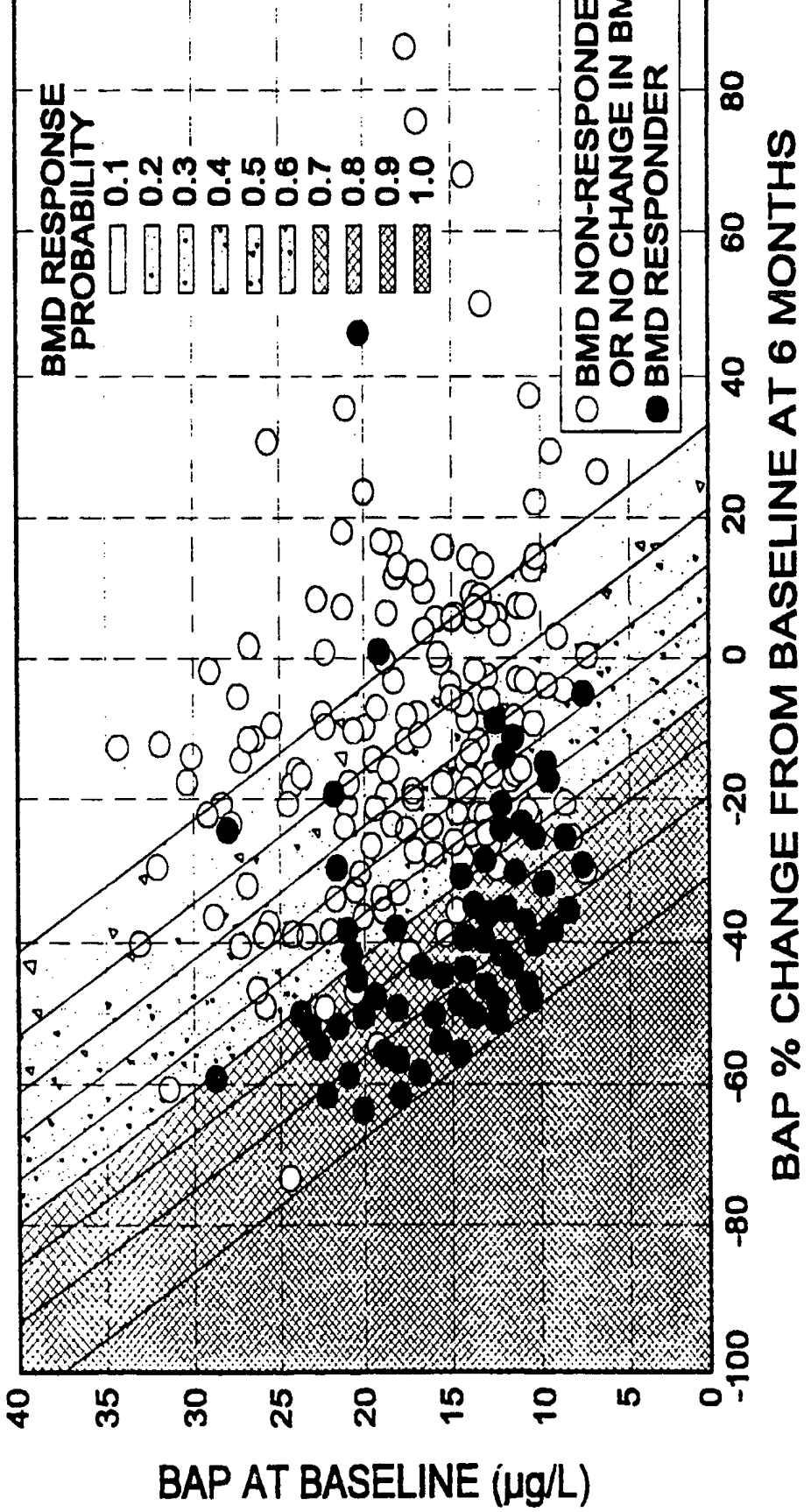
BMD RESPONSE PROBABILITY
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
BMD NON-RESPONDER OR NO CHANGE IN BM
BMD RESPONDER
BAP % CHANGE FROM BASELINE AT 6 MONTHS
80
60
40
20
0
-20
-40
-60
-80
-100
BAP AT BASELINE (µg/L)
40
35
30
25
20
15
10
5
0

Fig. 5

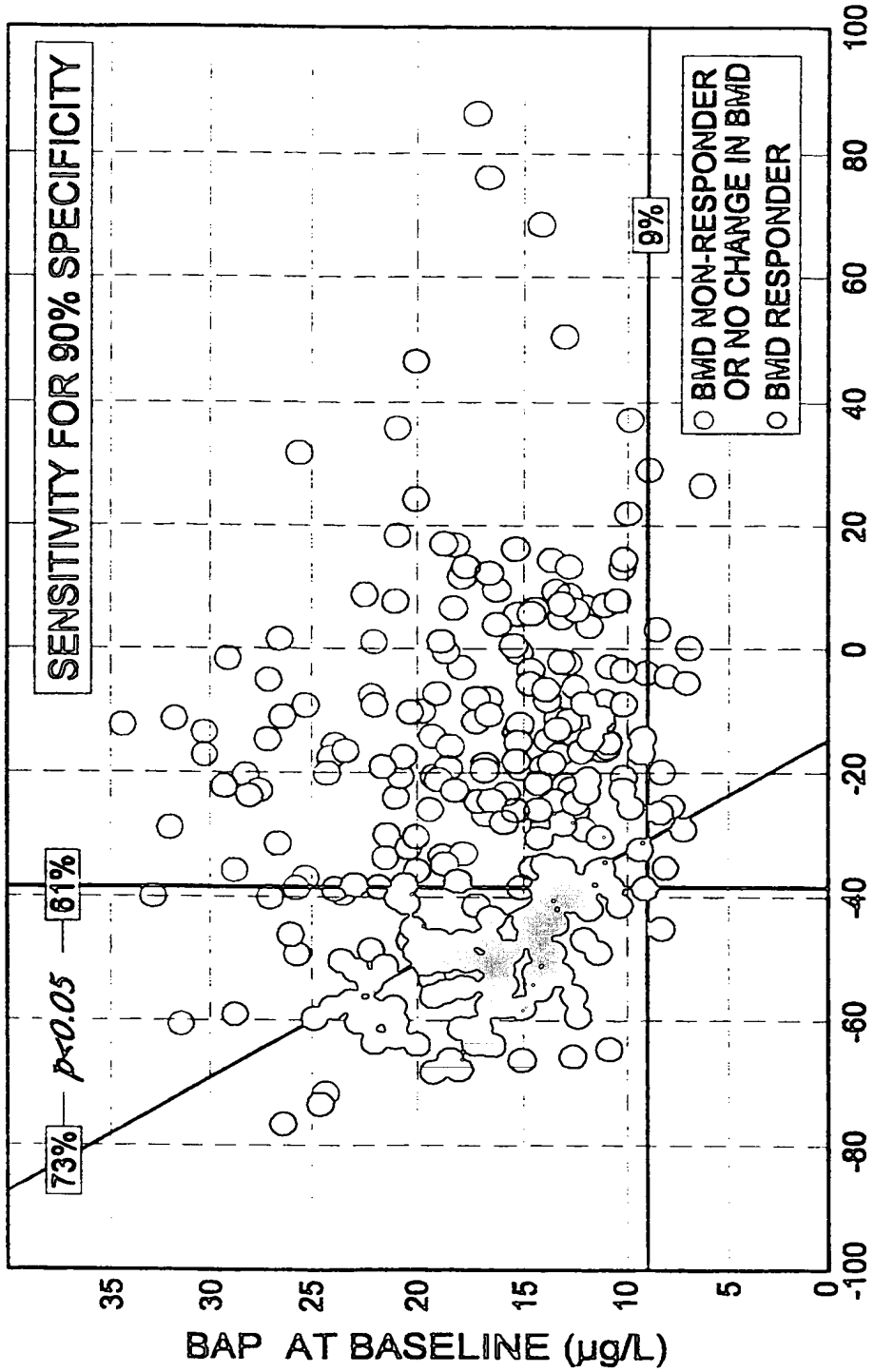
SENSITIVITY FOR 90% SPECIFICITY
BMD NON-RESPONDER OR NO CHANGE IN BMD
BMD RESPONDER
9%
61%
73%
p<0.05
BAP % CHANGE FROM BASELINE AT 6 MONTHS
BAP AT BASELINE (µg/L)
100
80
60
40
20
0
-20
-40
-60
-80
-100
35
30
25
20
15
10
5
0

Fig. 6

INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
PCT/US 99/20698

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MEDLINE, Washington DC USA; abstract no. 97320553, abstract XP002129136 cited in the application & C.J. ROSEN ET AL.: " The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement or calcium supplementation " JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, New York NY USA	1,28
Y	--- -/--	1-42

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	15/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Van Bohemen, C

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DOCID: <WO___0022437A1 IA>

INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 99/20698

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 124, no. 8, 19 February 1996 (1996-02-19) Columbus, Ohio, US; abstract no. 76765, XP002129138 abstract & P. ALEXANDERSEN ET AL.: " The effect of menopause and hormone replacement therapy on bone alkaline phosphatase" SCAND. J. CLIN. LAB. INVEST. , vol. 55, no. 7, 1995, pages 571-576, Copenhagen DK	1-42
A	BIOLOGICAL ABSTRACTS, Philadelphia PA USA; abstract no. XP002129137 & N. NIELSEN ET AL.: "Estimation of effects of salmon calcitonin in established osteoporosis by biochemical bone markers " CALCIFIED TISSUE INTERNATIONAL, vol. 55, no. 1, 1994, pages 8-11, New York NY USA	1-42

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